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(54) Title: METHODS AND COMPOSITIONS USING CELLULAR ASIALODETERMINANTS AND GLYCOCONJUGATES FOR TARGETING CELLS TO TISSUES AND ORGANS

(57) Abstract: The present invention is directed to methods for delivering cells to a target tissue in a mammal using glycoconjugate to traffic the cell to a desired organ in the mammal. The methods according to the present invention are especially applicable to administering lymphoid cells such as natural killer (NK) cells activated with interleukin-2 (IL-2), lymphokine-activated killer (LAK) cells and/or tumor-infiltrating lymphocytes (TILs) and/or cytotoxic lymphocytes (CTLs), or stem cells such as those derived from the bone marrow or from umbilical cord tissue. The methods are also useful for targeting a gene of interest to a tissue in a mammal by introducing a cell containing the gene of interest and administering a glycoconjugate to the mammal.

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TITLE OF THE INVENTION

METHODS AND COMPOSITIONS USING CELLULAR ASIALODETERMINANTS AND GLYCOCONJUGATES FOR TARGETING CELLS TO TISSUES AND ORGANS

This application claims the benefit of U.S. Provisional Patent Application No. 60/364,498, filed March 15, 2002, the entirety of which is incorporated by reference herein for all purposes.

FIELD OF THE INVENTION

The present invention is in the field of clinical medicine and therapy. The invention relates to methods and compositions for targeting cells to an organ of interest, using sialo- or asialodeterminants, particularly neoasialodeterminants, on cell surfaces and/or on free glycoconjugates.

BACKGROUND OF THE INVENTION

Morell et al. determined that when a sialyl group of ceruloplasmin is removed by neuraminidase, this plasma protein rapidly disappears from serum. They disclosed that this phenomenon is due to the uptake by the asialoglycoprotein (ASGP) receptor present in liver cells (*J. Biol. Chem., 243:*155 (1968)). Thereafter, it was reported that the ASGP receptor is present only in liver cells (*Adv. Enzymol., 41:*99, (1974)). Such specific uptake by liver cells has been identified from the fact that when asialoceruloplasmin or asialoorosomucoid, which is experimentally labeled with tritium, is injected into the living body, the isotope is selectively detected only in liver cells. Scheinberg, I. H., et al., Hepatic removal of circulating proteins, *in* Davidson C. S., *ed. Problems in Liver Diseases*, pp. 279-285, New York, Stratton Company, (1979). In addition, it was also disclosed that this receptor specifically recognizes and absorbs

glycoproteins having D-galactose or N-acetylgalactosamine as the terminal sugar group (*Ann. Rev. Biochem. 51*:531, (1982)).

The cell membrane of liver cells comprises a cell structure which combines with asialoglycoprotein terminated with galactose. This cell structure was first named hepato-binding protein (HBP) but is presently called asialoglycoprotein (ASGP) receptor. Further, it has been observed that among various desialylated glycoproteins, the desialylated alpha(1)-acid glycoprotein, asialoorosomucoid, most rapidly disappears from the serum after injection. Therefore, it has been determined that asialo-alpha(1)-acid glycoprotein is both specifically and well taken up by liver cells (*J. Biol. Chem., 245:*4397 (1970)). The ASGP receptor is constituted with a single polypeptide having a molecular weight of about 40,000 and can recognize a glycoprotein having a galactose residue at the nonreductive terminal position of the saccharide chain (i.e., asialoglycoprotein).

While the physiological functions of an ASGP receptor are still uncertain, it is believed that an ASGP receptor participates in the metabolism of glycoproteins. In fact, the increase of the blood level of an ASGP is observed in case of hepatic diseases such as chronic hepatitis, liver cirrhosis and hepatic cancer. Further, the decrease of the quantity of an ASGP receptor is observed in an experimental model of hepatic disorder induced by administration of chemicals.

In view of these phenomena, it may be possible to diagnose hepatic diseases through assessment of the quantity and quality of an ASGP receptor determined by the use of an ASGP-like substance, i.e., an ASGP receptor-directing compound. In fact, asialoglycoconjugates have been covalently linked to other agents as a means of targeting chemical (immunosuppressive drugs) and biological agents (antibodies) to be taken up by the liver for therapeutic and diagnostic purposes (*see, e.g.*, US Patent Nos. 5,346,696, 5,679,323, and 5,089,604).

Adoptive cellular immunotherapy in general is a treatment that employs biological reagents to effect an immune-mediated response. Currently, most adoptive immunotherapies are autolymphocyte therapies (ALT) directed to treatments using the patient's own immune cells which have been processed to either enhance the immune cell mediated response or to recognize specific antigens or foreign substances in the body, including cancer cells. The treatments are accomplished by removing the patient's lymphocytes and exposing these cells *in vitro* to biologics and drugs to activate the immune function of the cells. Once the autologous cells are activated, these *ex vivo* activated cells are reinfused into the patient to enhance the immune

system to treat various forms of cancer, infectious diseases, autoimmune diseases or immune deficiency diseases.

Adoptive immunotherapies may utilize, for instance, natural killer (NK) cells activated with interleukin-2 (IL-2), lymphokine-activated killer (LAK) cells and/or tumor-infiltrating lymphocytes (TILs) and/or cytotoxic lymphocytes (CTLs). LAK therapy involves the in vitro generation of LAK cells by culturing autologous peripheral blood leukocytes in high concentrations of IL-2. The LAK cells are then reinfused into the cancer patient in a treatment that may also involves infusion of IL-2. Rosenberg, et al., "Cancer immunotherapy using interleukin-2 and interleukin-2 activated lymphocytes," *Annual Review of Immunology 4*:681-709 (1986). TIL therapy involves the generation of LAK cells from mononuclear cells originally derived from the inflammatory infiltrating cells present in and around solid tumors, obtained from surgical resection specimens. Rosenberg, et al., "A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes," *Science 233*:1318-1321 (1986). Many further variations of adoptive immunotherapy have been developed in recent years. *See, e.g.*, U. S. Patent No, 6,406,699, issued June 18, 2002 to Wood, disclosing and claiming a composition and method of cancer antigen immunotherapy, and methods in references disclosed and cited therein.

In addition to cancer immunotherapies, adoptive immunotherapy has applications for deficiency or dysfunction of T cells associated with several diseases and conditions, including recurrent infections by viruses such as herpesvirus (HSV, VZV, CMV), hepatitis B virus, and papillomavirus. *See, e.g.,* Spiegel, R. J., "The alpha interferons: Clinical overview", *Seminars in Oncology 14*:1 (1987). ALT is also being evaluated in the treatment of patients infected with HIV. O. Martinez-Maza, "HIV-Induced Immune Dysfunction and AIDS-Associated Neoplasms," *in Biological Approaches to Cancer Treatment: Biomodulation*, M. Mitchell, Editor, McGraw-Hill, Inc., Chapter 9, pages 181-204 (1993).

A stem cell is a special kind of cell that has a unique capacity to renew itself and to give rise to specialized cell types. Although most cells of the body such as heart cells or skin cells, are committed to conduct a specific function, a stem cell is uncommitted and remains uncommitted until it receives a signal to develop into a specialized cell. In 1998, stem cells from early human embryos were first isolated and grown in culture. It is recognized that these stem cells are, indeed, capable of becoming almost all of the specialized cells of the body. In recent years, stem cells present in adults also have been shown to have the potential to generate replacement cells for a broad array of tissues and organs, such as the heart, the liver, the pancreas, and the nervous system. Thus, this class of adult human stem cell holds the promise of

being able to repair or replace cells or tissues that are damaged or destroyed by many devastating diseases and disabilities. It is highly useful to effect such therapies by targeting stem cells to particular organs of the body.

In the prior art, lymphocytes and stem cells generally have been presented to the desired organs either by injection into the tissue or by infusion into the local circulation. However, localization of normal bone marrow stem cells and lymphocytes to the liver has been demonstrated upon injection of such cells into mice. Samlowski et al., *Immunol. 88:*309-322 (1984); Samlowski et al., *Proc. Natl. Acad. Sci. 82:*2508-2512 (1985).

It is also known that a large proportion of cells infused into mammals adhere to the lung endothelium, independent of cell type or physiological homing properties. It has been observed that stem cells accumulate in the lungs when they are administered. Morrison et al., *Nature Medicine 2*:1281-1282 (1996); Martino et al., *Eur. J. Immunol. 23*:1023-1028 (1993); Pereira et al., *Proc. Natl. Acad. Sci. USA 92*:4857-4861(1993); and Gao et al., *Cells Tissues Organs 169*:12-20 (2001).

Orosomucoid, asialo-orosomucoid and agalacto/asialo-orosomucoid have been shown to inhibit neutrophil activation, superoxide anion generation, and platelet activation. Costello et al., *Clin Exp Immunol 55:*465-472 (1984); and Costello et al., *Nature 281:*677-678 (1979). These proteins also induced transient immunosuppression and protected against TNF challenge. Bennett, et al., *Proc. Natl. Acad. Sci. USA 77:*6109-6113 (1980) and Libert, et al., *J. Exp. Med. 180:*1571-1575 (1994). Orosomucoid demonstrated specific binding to pulmonary endothelial cells, which appeared to be independent of carbohydrate recognition sites. Schnitzer, et al., *Am. J. Physiol 263:*H48-H55 (1992). Moreover, orosomucoid was shown to bind to skin capillary endothelial cells in a dose dependent manner, thereby maintaining normal capillary permeability in the face of inflammatory agonists that caused leakage in control animals. Muchitsch, et al., *Arch Int Pharmacodyn 331:*313-321 (1996). Similarly, infused orosomucoid bound to kidney capillaries and restored the permselectivity of glomerular filtration. Muchitsch, et al., *Nephron 81:*194-199 (1999).

Entrapment of neuraminidase-treated lymphocytes in the liver also has been reported, including autoimmune reactions against liver cells by syngeneic neuraminidase-treated lymphocytes, in mice intravenously injected with lymphocytes isolated from spleen or thymus. Kolb-Bachofen, V., et al., *Immunol. 123*:2830-2834 (1979). Studies on interactions between neuraminidase-treated rat lymphocytes and liver cells in culture have demonstrated adhesion

between cells is due to stereo-specific interactions between a mammalian hepatic membrane lectin (i.e., the ASGP receptor) and galactosyl residues which are exposed on the lymphocyte surface after removal of sialic acid residues. Kolb, H., et al., *Adv. Exp. Med. Biol. 114*:219-222 (1979).

In view of the above, a need exists to develop methods for delivery of lymphocytes and stem cells through the circulation to specific organs. Such methods would provide a means to target non-invasively solid organs such as the liver, heart, lungs and kidneys. In addition, very diffuse tissues, such as the lung, which are not amenable to dosage by injection could be targeted. Such methods would be useful in adoptive immunotherapies and regenerative stem cell therapies involving such organs as the liver, heart, lungs and kidneys.

The present invention addresses these and other needs.

BRIEF SUMMARY OF THE INVENTION

The present invention features a method for delivering a cell to a target tissue in a mammal comprising the steps of administering a carbohydrate presenting molecule *(e.g.,* a glycoconjugate) to a mammal and then administering the cell to the mammal.

As used herein, the term "administering" refers to any method of inducing an increased concentration of the cell in the circulation of the mammal, whether by infusion from an extraneous source or by mobilizing the cell into the circulation from a depot within the mammal, such as the marrow. Means for mobilizing stem cells, for instance, using GM-CSF and GCSF, for example, are well known in the art. *See, e.g.,* Simmons et al., The mobilization of primitive hemopoietic progenitors into the peripheral blood. *Stem Cells, 12 Suppl 1:*187-201 (1994).

The methods according to the present invention are especially applicable to stem cells, such as those derived from the bone marrow, peripheral blood, umbilical cord or from mesenchymal stem cells expanded in culture. The stem cells within the scope of the invention include any cell capable of differentiating into a desired target tissue. Such cells include pluripotent stem cells, embryonic stem cells, multipotent adult stem cells, and progenitor or precursor cells.

The methods according to the present invention also are especially applicable to immune system cells, such as natural killer (NK) cells activated with interleukin-2 (IL-2), lymphokine-activated killer (LAK) cells and/or activated lymphocytes including but not limited to tumor-infiltrating lymphocytes (TILs).

The methods of the present invention allow cells such as normal stem or immune cells to be targeted to such target tissues as the heart, the liver, the kidneys and the lungs, among others. In some embodiments wherein the cell is targeted to the heart, the methods feature administering an orosomucoid (O) or administering an asialoorosomucoid (ASO), and administering the cell to the mammal. In embodiments wherein the cell is targeted to the lungs, the methods feature administering the cell to the mammal in a saline or a serum albumin-saline solution or cell culture media without protein/albumin. In embodiments wherein the cell is targeted to the liver, the methods feature administering an orosomucoid or an asialoorosomucoid and administering the cell to the mammal. In some embodiments, the orosomucoid is administered concurrently or prior to administering the cell to the mammal. The methods according to the present invention are also useful for either inhibiting or enhancing sequestration of a stem cell or immune cell in the liver of a mammal even in the absence of targeting the cell to a target organ.

The glycoconjugates of the present invention may be generally represented by the general formula P-(S)x-Gal wherein P is a peptide residue of a human serum glycoprotein and S is a sugar residue of a human serum glycoprotein; x is an integer from 1 to 100 and Gal is galactose residue. The glycoconjugates may be partially or completely asialylated. Especially useful glycoconjugates include fetuins, asialofetuins, orosomucoids and asialoorosomucoids.

The glycoconjugates may be administered to the mammal in any time frame relative to administering the cell. They may be administered before, after or simultaneously with the administration of the cell. In a typical embodiment, the glycoconjugates are administered prior to the cell. The glycoconjugates and the cell may be administered via any suitable route. In preferred embodiments, they are administered parenterally, and more preferably, intravenously to the mammal.

The methods according to the present invention are also useful for targeting a gene of interest to a tissue in a mammal by introducing a cell naturally containing, or a cell transformed with, the gene of interest to the mammal. Such methods are useful for treating a disease characterized by a deficiency in a gene product in a mammal by administering a cell comprising a functional gene encoding the gene product into the mammal and administering a glycoconjugate to the mammal. According to these methods, a cell containing an exogenous functional gene of interest may be administered and localized to a particular organ in the body where it can function to produce a deficient gene product.

Also, the methods according to the present invention are useful for treating a disease characterized by tissue damage in a mammal by administering a cell and administering a glycoconjugate to the mammal. Because stem cells have the potential to generate replacement cells for a broad array of tissues and organs, such as the heart, the pancreas, and the nervous system, stem cells may be targeted to particular organs in the body to repair or replace cells or tissues that are damaged or destroyed by many devastating diseases and disabilities. In some embodiments, the disease may be a heart disease, a lung disease, a kidney disease or a liver disease, for example, myocardial infarction, emphysema, cystic fibrosis, microalbuminuria, nephritis, stroke or hepatitis.

The methods according to the present invention are also useful for treating a disease characterized by tissue damage in a mammal by administering a glycoconjugate to the mammal and administering chemicals or biopharmaceuticals that mobilize stem cells into the circulation. The concentration of circulating mobilized stem cells may be limited because certain organs may sequester stem cells, thereby limiting delivery of an effective dose to the damaged organ. By inhibiting sequestration, the glycoconjugates of the invention increase the cell dose at the organ; thereby increasing the potential to generate replacement cells. The methods including agents to mobilize stem cells also can be used for a broad array of tissues and organs, such as the heart, the pancreas, and the nervous system. Mobilized stem cells may be targeted to particular organs in the body to repair or replace cells or tissues that are damaged or destroyed by many devastating diseases and disabilities. In some embodiments wherein stem cells are mobilized, the disease may be a heart disease, a lung disease, a kidney disease, a neurological disease or a liver disease such as, for example, myocardial infarction, emphysema, cystic fibrosis, microalbuminuria, nephritis, stroke or hepatitis.

In other embodiments, the present invention provides pharmaceutical compositions comprising a cell and a glycoconjugate, *e.g.,* glycoprotein. Glycoproteins useful in the present invention include, for example, fetuins, orosomucoids (O) and asialoorosomucoids (ASO). In other aspects, the present invention features an article of manufacture, comprising packaging material and a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent comprises a glycoconjugate of the invention that is therapeutically effective for targeting a cell to a desired organ according to the present invention, and wherein the packaging material comprises a label which indicates that the pharmaceutical agent can be used for targeting a cell to a desired organ according to the present invention. In some embodiments, the article of manufacture further comprises additional reagents, such as solutions for making cell suspensions to be administered, and/or printed instructions, for use in targeting cells according to

the invention. Such articles include, for instance, kits for treating tissue damage or for delivering a functional gene or gene product to a tissue in a mammal comprising a cell and a glycoprotein. Glycoproteins useful in the articles of manufacture of the invention include fetuins, asialofetuins, orosomucoids and asialoorosomucoids.

In still other embodiments, the present invention provides methods for derivatization of stem cell or lymphoid cell populations to generate an asialodeterminant-bearing cell preparation to facilitate hepatic entrapment. In particular, the invention provides derivatized, activated stem cells or lymphocytes that have asialadeterminants on their surface that have been generated by enzymatic or chemical means so that these cells, when administered parenterally, circulate, are bound, and sequestered or entrapped by the liver via the ASGP receptor. Methods for treating whole viable cells with a sialidase, such as neuraminidase, are known in the art. *See, e.g.,* Neubauer, R. H., et al., Identification of normal and transformed lymphocyte subsets of nonhuman primates with monoclonal antibodies to human lymphocytes. *J. Immunol. 130*:1323-1329 (1983); Kolb-Bachofen, V., et al., 1979, *supra*; and Kolb, H., et al., 1979, *supra*.

For instance, the invention provides a process of derivatization of stem cells to generate neoasialadourminants on the surface of such calls, for the purpose of directing these tells to the liver to repair or regenerate liver functions and structures, or for delivery of normal genes or genetically engineered cells for the purpose of curing or ameliorating disease states. Operative elements of this aspect of the invention are the ability to direct the localization of the transfused stem cells bearing artificially created neoasialoglycodeterminants, their ability to create a micro-chimera of the recipient, and the mechanism by which of the neodeterminants are specifically sequestered by the liver. Thus, assimilation of these neoasialoglycodeterminant-bearing cells would result in microchimerism (a mixture of derivatized stem cells and the original host cells that were genetically abnormal. The modified stem cells would express at least the minimum required amount of the abnormal or missing protein or regulatory function needed for reversing or ameliorating the disease phenotype. The modified stem cells could be derived from patient's blood, bone marrow or other stem cell-producing organ such as adipose tissue, or may be derived from another individual or a stem cell line.

The invention also provides methods for manipulation of *in vivo* cell trafficking patterns of lymphoid cytolytic cells by specifically facilitating the hepatic sequestration of parenterally administered activated lymphocytes by derivitizing the cell surface with enzymes that generate "neoasialodeterminants". Thus, activated lymphoid populations have cell surface asialodeterminants capable of binding to the ASGP receptor, and this binding can be further enhanced by enzymatic treatments that generate new cell surface asialodeterminants.

These methods may be used, for instance, to improve the efficiency of adoptive immunotherapy for liver metastasis or primary liver tumors by facilitating hepatic entrapment (via the ASGP receptor) of parenterally administered cells that have been derivatized to generate cell surface asialodeterminants. Metastasis of various cancers to the liver are difficult to treat. For example, elimination of breast cancer metastases to liver must be achieved prior to harvesting of bone marrow or autologous stem cell products for transplantation. Chemotherapy alone can take months to achieve a complete response. It often leads to bone marrow suppression making the harvesting of stem cells from individuals extremely difficult. In cases where the tumor is chemotherapy resistant, very few therapeutic options remain. Adoptive immunotherapies do exist in which the patients own cells can be "educated" in culture to recognize the tumor and then these cells are transferred to the patient intravenously to find and destroy the tumor.

If the tumor burden is primarily in the liver, it may be useful to have several cycles of therapy directed specifically toward the elimination of tumor from the liver. This can be accomplished according to the present invention, by treating the activated lymphoid populations (that have been grown or "educated" in culture) with enzymes or other treatments that include (but are not limited to sialidases, such as neuraminidases, that modify the cell surface glycosylation sites to expose asialodeterminants. The number of these determinants are thereby dramatically increased and hence the modified cells bind to hepatic ASGP receptors more readily and dissociate less frequently than cells bearing the "normal" number of asialodeterminants.

Assimilation of neoasialoglycodeterminant-bearing lymphoid cells results in microchimerism, as described for stem cells above, a mixture of infused lymphocytes that have or have not been genetically engineered with the original host lymphoid cells that exist at the site. The infused lymphoid cells would augment or enhance the immune response by dividing and entering the circulation and recruiting other cell populations to participate in the local immune response. The hepatic environment is ideally suited for the development of immune responses due to the presence of cells of the innate immune system as well as professional antigen presenting cells in the sinusoids and vasculature, particularly the portal system.

For example, several studies have shown that responses to metastatic cutaneous melanoma, for instance, can be achieved using regional administration to the liver of activated lymphocytes. *See, e.g.,* Keilhoiz, U. et al., Regional adoptive imunotherapy with interleukin-2 and lymphokine-activated killer (LAK) cells for liver metastasis, *Eur. J. Cancer 3OA*:103-105 (1994). The invention methods of using activated lymphocytes that have been modified to generate additional cell surface asialodeterminants permits the delivery of activated lymphocytes

to the liver regionally, via the hepatic artery or portal vein or peripheral vein, without the use of invasive procedures to deliver these cells to a primary hepatic or non-hepatic tumor, or to metastatic lesions distant from a primary hepatic or non-hepatic cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 provides a schematic of liver entrapment of bone marrow stem cells and lymphocytes in the liver. Asialoglycodeterminants on the surface of cells react with ASGP receptors on the surface of hepatocytes resulting in the localization of the bone marrow stem cells and the lymphocytes in the liver. Glycoconjugates including asialoglycoconjugates block such interactions between asialoglycodeterminants on the surface of cells with ASGP receptors on the surface of hepatocytes.

FIGURE 2 shows the carbohydrate structure on two exemplary glycoproteins of the invention.

FIGURE 3 shows the relative binding affinities of different carbohydrates for the ASGP receptor.

FIGURE 4 shows the relative binding affinities of different carbohydrates for the ASGP receptor.

FIGURE 5 shows a schematic of an experimental system for studying adherence of NK/LAK cells to monolayer cultures of (1) a human hepatoma cell line (HEP2G), an asialoglyprotein repetor positive (ASGPR+) cell line that exhibits minimal deviation from cells in human liver tissue, and (2) a human renal cell carcinoma cell line (CAKI-2), an ASGPR— cell line.

FIGURE 6 shows a plot of results of testing effects of asialofetuin (ASF) and fetuin (F) on adherence of NK/LAK cells (as represented by NK/LAK activity) to HEP2G monolayers at 4° C. LAK activity (50%) adheres to human minimal deviation hepatoma, HEPG2, at 4° C, in the presence of the control fully sialylated protein, fetuin, F (LAK-NA/F). LAK activity does not adhere to the HEPG2 monolayer in the presence of asialofetuin, ASF (LAK-NA/ASF). LAK cells were incubated in the presence of ASF alone, i.e.,no adherence to monolayer not performed (LAK/ASF). CONTROL cells did not kill RAJI targets (CONTROL). ** This is representative of three different donors.

FIGURE 7 shows results of testing effects of asialofetuin (ASF) and fetuin (F) on adherence of NK/LAK cells to HEP2G and CAKI-2 cells at 23° C. The effector cell populations were: an untreated 3-day old LAK preparation (LAK) and the same population treated with

Vibrio cholera neuraminidase (LAK/NS). LAK adherence to HEPG2 (ASGPR+) and CAKI-2 (ASGPR-) in the presence of either ASF or F assayed on K562. (LAK=3 day LAK; K5 = K562 targets; FET = fetuin; ASF = asialofetujn; LAK/CAKI/ASF/K5 = LAK, adherence on CAKI pretreated with ASF, assayed on K562).

FIGURE 8 shows additional results of testing effects of asialofetuin (ASF) and fetuin (F) on adherence of NK/LAK cells to HEP2G and CAKI-2 cells at 23° C, as in Figure 7. Adherence of neuraminidase-treated LAK to HEPG2 (ASGPR+) and CAKI-2 (ASGPR-) in the presence of ASF or F. (LAK/NS = neuraminidase-treated LAK; EXAMPLE- LAK/CAK/NS/ASF/K5 = neuraminidase-treated LAK, adherence on CAKI pretreated with ASF, assayed on K562).

FIGURE 9 shows additional results of testing effects of asialofetuin (ASF) and fetuin (F) on adherence of NK/LAK cells to HEP2G and CAKI-2 cells at 23° C, as in Figure 7. LAK adherence to HEPG2 (ASGPR+) and CAK.I-2 (ASGPR-) in the presence of either ASF or F assayed on RAJI. (LAK = 3 day LAK; R= RAJI targets; FET = fetuin; ASF = asialofetujn; LAK/CAKI/ASF/R = LAK, adherence on CAKI pretreated with ASF, assayed on RAJI).

FIGURE 10 shows additional results of testing effects of asialofetuin (ASF) and fetuin (F) on adherence of NK/LAK cells to HEP2G and CAKI-2 cells at 23° C, as in Figure 7. Adherence of neuraminidase-treated LAK to HEPG2 (ASGPR+) and CAKI-2(ASGPR-) in the presence of ASF or F.(LAK/NS = neuraminidase-treated LAK; EXAMPLE- LAK/CAKI/NS/ASF/R = neuraminidase-treated LAK, adherence on CAKI pretreated with ASF, assayed on RAJI).

FIGURE 11 shows results of testing effects of cell surface modifications on adherence of NK/LAK cells to HEP2G cell monolayers. Cytotoxic activity of 5-day LAK, Neuramindase-treated LAK, and Control (no IL-2), assayed on K562.

FIGURE 12 shows additional results of testing effects of cell surface modifications on adherence of NK/LAK cells to HEP2G cell monolayers, as in Figure 11. Cytotoxic activity of 5-day LAK, Neuramindase-treated LAK, and Control (no IL-2), assayed on RAJI cells.

FIGURE 13 shows additional results of testing effects of cell surface modifications on adherence of NK/LAK cells to HEP2G cell monolayers, as in Figure 11. Adherence of LAK activity to HEPG2 (ASGPR+) after cell surface modification with neuraminidase, 2,3- or 2,6-sialyltransferases. (EXAMPLE: LAK/HEP/NASE/K5 = neuraminidase- treated LAK adhered to HEPG2 assayed on K562).

FIGURE 14 shows additional results of testing effects of cell surface modifications on adherence of NK/LAK cells to HEP2G and CAKI-2 cell monolayers, as in Figure 11. Adherence

of LAK activity to CAKI-2 (ASGPR-) after cell surface modification with neuraminidase, 2,3- or 2,6-sialyltransferases. (Dotted lines in both Figures 13 and 14 are the same controls.)

FIGURE 15 shows additional results of testing effects of cell surface modifications on adherence of NK/LAK cells to cell HEP2G and CAKI-2 monolayers, as in Figure 11. Adherence of LAK activity to HEPG2 (ASGPR+) after cell surface modification with neuraminidase, 2,3- or 2,6-sialyltransferases, assayed on RAJI cells.

FIGURE 16 shows additional results of testing effects of cell surface modifications on adherence of NK/LAK cells to cell HEP2G and CAKI-2 monolayers, as in Figure 11. Adherence of LAK activity to CAKI-2 (ASGPR-) after cell surface modification with neuraminidase, 2,3- or 2,6-sialyltransferases, assayed on RAJI cells.

DETAILED DESCRIPTION OF THE INVENTION

A. Introduction

The present invention is directed to methods for delivering a cell to a target tissue in a mammal. The methods comprise the steps of administering, either simultaneously or sequentially, a carbohydrate presenting molecule *(e.g.,* glycoconjugate) and a cell to the mammal. In the methods of the present invention, glycoconjugates, especially asialoglycoconjugates, including asialo plasma proteins such as asialoorosomucoid (asialo alpha-(1)-acid glycoprotein), are thought to transiently bind the hepatic ASGP receptor and thereby competitively inhibit attachment of cells bearing asialodeterminants from these receptors. Without wishing to be bound by theory, hyposialylated and desialylated proteins/glycoconjugates (also called asialoglycoconjugates) and cells which bear similar determinants are bound or "trapped" in the liver as a consequence of binding to the hepatic ASGP receptors *(see,* Figure 1). Occupation of the receptor by the asialoglycoconjugate inhibits sequestration of the cells bearing similar determinants of interest in the liver.

In addition, the present disclosure shows that glycoconjugates of the invention prevent infused cells from concentrating in the alveolar vasculature. This finding suggests that lung sequestration of the cells may be related to expression of inflammatory receptors on endothelial cells, analogous to the reperfusion syndrome *(see, e.g., Kilgore et al. Cardiovasc Res 28:437-444 (1994)* and *Eror et al., Clin Immunol 90:266-275 (1999).* This is supported by reports that orosomucoid, ASO and agalacto/asialo-orosomucoid inhibit neutrophil activation superoxide anion generation, as well as platelet activation as noted above.

The present invention further demonstrates that the glycoproteins may be used to traffic or target cells to particular organs of the body by altering the particular glycoconjugate administered. The present methods are useful to improve the efficacy of bone marrow and stem cell transplants, tissue repair, gene therapy or adoptive immunotherapies.

In embodiments wherein the cell is targeted to the lungs, the methods feature administering the cell to the mammal in a saline or serum albumin-saline solution. In some embodiments wherein the hematopoietic stem cell is targeted to the heart, the methods feature administering an asialoorosomucoid, and administering the cell to the mammal. In other embodiments wherein the mesenchymal stem cell is targeted to the heart, the methods feature administering an orosomucoid, and administering the cell to the mammal. In embodiments wherein the hematopoietic stem cell is targeted to the liver, the methods feature administering an orosomucoid and administering the cell to the mammal. In other embodiments wherein the mesenchymal stem cell is targeted to the liver, the methods feature administering an asialoorosomucoid and administering the cell to the to the mammal. In some embodiments, the orosomucoid or asialoorosomucoid is administered in at least two infusions prior to administering the cell to the mammal. The methods according to the present invention are also useful for inhibiting sequestration of a cell in the liver of a mammal even in the absence of targeting the cell to a target organ.

Asialoglycoconjugates, for example, asialofetuin and other asialo plasma proteins, are able to bind to the hepatic parenchyma and Kupffer cell ASGP receptors. Blocking these receptors from binding and trapping cells bearing asialodeterminants, such as bone marrow cells, facilitates and increases the interval of their systemic circulation. In the case of bone marrow stem cells, the administration of these compounds prevents the loss and destruction of bone marrow stem cells and increases the efficiency of engraftment. Bone marrow cells have cell surface asialodeterminants capable of binding to the ASGP receptor, and this binding can be inhibited by the application of ASGPs.

The present invention takes advantage of the observation that when human peripheral hematopoietic stem (CD34+) cells or mesenchymal stem cells are infused into the jugular vein of immunodeficient mice, they localize predominantly in the lungs. When the cells are preceded by an infusion of asialoorosomucoid, the hematopoietic stem cells predominantly localize in the heart, whereas the mesenchymal stem cells localize in the liver. Alternately, when the cells are preceded by an infusion of orosomucoid (O), the hematopoietic stem cells localize in the liver, whereas the mesenchymal stem cells predominantly localize in the heart.

These protein infusions cause a more quantitative localization into the specific organs than occurs without them. Furthermore, hematopoietic stem cells that localize in the heart due to the influence of asialoorosomucoid leave the vascular space and are observed among the cardiac muscle cells by one hour after infusion. Moreover, once in the tissue, these cells lose their CD34 antigen, indicating that they are in the process of differentiating into cardiomyocytes or heart components (e.g., blood vessels). Additionally, at one hour CD34+ cells have been demonstrated to move from the vasculature into lung tissue. In an orosomucoid-treated mouse, clusters of stem cells are found in the liver parenchyma and are also demonstrated to lose their CD34 antigen, again suggesting differentiation into hepatocytes/hepatic or liver parenchyma.

The present invention demonstrates the ability to direct high concentrations of stem cells to a specific organ in an atraumatic manner. This enhances the probability and the rate at which stem cells migrate into a target tissue and differentiate into the desired cell type. The present invention utilizes the observation that delivery of orosomucoid or ASO to the vessel proximal to the heart causes transfused stem cells to accumulate in the heart. Without wishing to be bound by theory, the effect may be caused by the glycoprotein infusion sensitizing the endothelium directly downstream from the infusion site, which causes the endothelial cells to bind stem cells and enhance their migration across the endothelium into the tissue.

The present findings with glycoconjugates indicate that the majority of a stem cell transfusion can be concentrated in the target organ, thereby providing the means to deliver an effective regimen of cell doses. This offers an opportunity to non-invasively target solid organs such as the heart, thereby competing with invasive direct injection. Perhaps more importantly, glycoconjugates provide the means to target very diffuse tissues, such as the liver and the kidney, which are not amenable to dosage by injection.

It is recognized that hematopoietic stem cells (HSC) recovered from the marrow, peripheral blood or umbilical cord blood and mesenchymal stem cells (MSC) recovered as marrow stromal cells, stromal cells from liposuction fat, or proliferated from stationary stromal progenitor cells in cord blood-depleted expelled placentas appear to be almost interchangeable in their differentiation ability, and act as multipotent stem cells.

Such cells have been shown to differentiate into functional cells when localized in specific organs and tissues: hepatocytes and cholangiocytes in the liver, cardiac muscle cells and arterial smooth muscle cells and endothelial cells in the heart, pneumocytes I & II in alveoli and bronchial epithelium in the lungs, chondrocytes for cartilage restoration, and intestinal mucosal cells, small, medium and large blood vessels in the heart, etc.

B. Stem Cells

Stem cells may hold the key to replacing cells lost in many devastating diseases such as Parkinson's disease, diabetes, acute and chronic heart disease, end-stage kidney disease, liver failure, and cancer. For many diseases, there are no effective treatments but the goal is to find a way to replace what natural processes have taken away.

To date, published scientific papers indicate that adult stem cells have been identified in brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, dental pulp of the tooth, retina, liver, and pancreas. Thus, adult stem cells have been found in tissues that develop from all three embryonic germ layers.

By way of definition, the following terms are understood in the art:

A "stem cell" is a cell from the embryo, fetus, or adult that has, under certain conditions, the ability to reproduce itself for long periods or, in the case of adult stem cells, throughout the life of the organism. It also can give rise to specialized cells that make up the tissues and organs of the body.

A "pluripotent stem cell" has the ability to give rise to types of cells that develop from the three germ layers (mesoderm, endoderm, and ectoderm) from which all the cells of the body arise. The only known sources of human pluripotent stem cells are those isolated and cultured from early human embryos and from fetal tissue that was destined to be part of the gonads.

An "embryonic stem cell" is derived from a group of cells called the inner cell mass, which is part of the early (4- to 5-day) embryo called the blastocyst. Once removed from the blastocyst the cells of the inner cell mass can be cultured into embryonic stem cells. These embryonic stem cells are not themselves embryos.

An "adult stem cell" is an undifferentiated (unspecialized) cell that occurs in a differentiated (specialized) tissue, renews itself, and becomes specialized to yield all of the specialized cell types of the tissue in which it is placed when transferred to the appropriate tissue. Adult stem cells are capable of making identical copies of themselves for the lifetime of the organism. This property is referred to as "self-renewal." Adult stem cells usually divide to generate progenitor or precursor cells, which then differentiate or develop into "mature" cell types that have characteristic shapes and specialized functions, e.g., muscle cell contraction or nerve cell signaling. Sources of adult stem cells include bone marrow, blood, the cornea and the retina of the eye, brain, skeletal muscle, dental pulp, liver, skin, the lining of the gastrointestinal tract and pancreas.

Stem cells from the bone marrow are the most-studied type of adult stem cells. Currently, they are used clinically to restore various blood and immune components to the bone marrow via transplantation. There are currently identified two major types of stem cells found in bone marrow: hematopoietic stem cells (HSC, or CD34+ cells) which are typically considered to form blood and immune cells, and stromal (mesenchymal) stem cells (MSC) that are typically considered to form bone, cartilage, muscle and fat. However, both types of marrow-derived stem cells recently have demonstrated extensive plasticity and multipotency in their ability to form the same tissues.

The marrow, located in the medullary cavity of bones, is the sole site of hematopoiesis in adult humans. It produces about six billion cells per kilogram of body weight per day. Hematopoietically active (red) marrow regresses after birth until late adolescence after which time it is focused in the lower skull vertebrae, shoulder and pelvic girdles, ribs, and sternum. Fat cells replace hematopoietic cells in the bones of the hands, feet, legs and arms (yellow marrow). Fat comes to occupy about fifty percent of the space of red marrow in the adult and further fatty metamorphosis continues slowly with aging. In very old individuals, a gelatinous transformation of fat to a mucoid material may occur (white marrow). Yellow marrow can revert to hematopoietically active marrow if prolonged demand is present such as with hemolytic anemia. Thus hematopoiesis can be expanded by increasing the volume of red marrow and decreasing the development (transit) time from progenitor to mature cell.

The marrow stromal consists principally of a network of sinuses that originate at the endosteum from cortical capillaries and terminate in collecting vessels that enter the systemic venous circulation. The trilaminar sinus wall is composed of endothelial cells; an underdeveloped, thin basement membrane, and adventitial reticular cells that are fibroblasts capable of transforming into adipocytes. The endothelium and reticular cells are sources of hematopoietic cytokines. Hematopoiesis takes place in the intersinus spaces and is controlled by a complex array of stimulatory and inhibitory cytokines, cell-to-cell contacts and the effects of extracellular matrix components on proximate cells. In this unique environment, lymphohematopoietic stem cells differentiate into all of the blood cell types. Mature cells are produced and released to maintain steady state blood cell levels. The system may meet increased demands for additional cells as a result of blood loss, hemolysis, inflammation, immune cytopenias, and other causes. The engraftment efficiency of bone marrow stem cells could be improved by preventing entrapment by the liver via the hepatic ASGP receptor.

A "progenitor or precursor" cell occurs in fetal or adult tissues and is partially specialized; it divides and gives rise to differentiated cells. Researchers often distinguish precursor/progenitor cells from adult stem cells in that when a stem cell divides, one of the two new cells is often a stem cell capable of replicating itself again. In contrast when a progenitor/precursor cell divides, it can form more progenitor/precursor cells or it can form two specialized cells. Progenitor/precursor cells can replace cells that are damaged or dead, thus maintaining the integrity and functions of a tissue such as liver or brain.

Means for isolating and culturing stem cells useful in the present invention are well known. Umbilical cord blood is an abundant source of hematopoietic stem cells. The stem cells obtained from umbilical cord blood and those obtained from bone marrow or peripheral blood appear to be very similar for transplantation use. Placenta is an excellent readily available source for mesenchymal stem cells. Moreover, mesenchymal stem cells have been shown to be derivable from adipose tissue and bone marrow stromal cells and speculated to be present in other tissues. While there are dramatic qualitative and quantitative differences in the organs from which adult stem cells can be derived, the initial differences between the cells may be relatively superficial and balanced by the similar range of plasticity they exhibit. For instance, adult stem cells both hematopoietic and mesenchymal, under the appropriate conditions can become cardiac muscle cells. Delineation of full range of potential for adult stem cells has just begun.

Stem cells may be isolated for transduction and differentiation using known methods. For example, in mice, bone marrow cells are isolated by sacrificing the mouse and cutting the leg bones with a pair of scissors. Stem cells may also be isolated from bone marrow cells by panning the bone marrow cells with antibodies which bind unwanted cells, such as CD4+ and CD8+ (T cells), CD45+ (panB cells), GR-1 (granulocytes), and lad (differentiated antigen presenting cells). For an example of this protocol see, *Inaba et al., I. Exp. Med.* 176-1693 1702(1992).

In humans, CD34+ hematopoietic stem cells can be obtained from a variety of sources including cord blood, bone marrow, and mobilized peripheral blood. Purification of CD34+ cells can be accomplished by antibody affinity procedures. An affinity column isolation procedure for isolating CD34+ cells is described by *Ho et al., Stem Cells 13 (suppl. 31: 100-105(1995)*. See also, *Brenner, Journal of Hematotherapy 2: 7-17 (1993)*. Methods for isolating, purifying and culturally expanding mesenchymal stem cells are known. Specific antigens for MSC are also known *(see,* US Patent Nos. 5,486,359 and 5,837,539).

C. Carbohydrate presenting molecule

The carbohydrate presenting molecules useful in the present invention can be any molecule capable of presenting the appropriate carbohydrate structure that leads to enhancing or inhibiting the targeting of the cell of interest to a target tissue. The targeting function can be carried out using a carbohydrate molecule such as an oligosaccharide, polysaccharide, or the carbohydrate structure can be bound to larger molecule or carrier, referred to here as a glycoconjugate. Typically, the carbohydrate molecule will be linked to either a naturally occurring carrier *(e.g.,* as part of a glycoprotein or glycolipid) or the carrier may be synthetic *(e.g.,* an engineered polypeptide sequence). One of skill will recognize that a number of carriers can be used to present the appropriate structure. Examples of appropriate carrier molecules include polypeptides, lipids, and the like. Preparation and use of targeted compounds using asialo carbohydrate moieties is described in the art *(see, e.g.,* US Patent Nos. 5,679,323, 5,089,604, 5,032,678 and 5,284,646). One of skill will recognize that such compounds can also be used as carbohydrate presenting molecules useful in the present invention.

In cases in which the glycoconjugate is a glycoprotein it may be generally represented by the general formula P-(S)x-Gal wherein P is a peptide residue of a human serum glycoprotein and S is a sugar residue of a human serum glycoprotein; x is an integer from I to 100 and Gal is a galactose residue. Especially useful glycoconjugates include fetuins and asialofetuins *(see,* Figure 2), orosomucoids and asialoorosomucoids and galactose-bonded polylysine, galactose-bonded polyglucosamine, and the like.

The methods of the present invention allow cells such as stem cells to be targeted to such target tissues as the heart, the liver, the kidneys and the lungs, among others. Parenteral administration of a glycoconjugate, such as asialoorosomucoid, may be used to block the hepatic ASGP receptor and allow the cells bearing surface asialodeterminants (for example, peanut agglutinin (PNA)+ cells) to continue to circulate and migrate to the marrow space. Asialoorosomucoid is one of the glycoproteins which has been shown to bind to the hepatic ASGP receptor and has been extensively used to characterize this receptor.

Different compounds have different binding affinities for the ASGP receptor, depending upon the carbohydrate presented *(see,* Figures 3 and 4). Thus, one of skill can modulate cell targeting by using compounds that present different carbohydrate structures.

Intravenous administration of a glycoconjugate, especially an ASGP such as asialoorosomucoid, may be used to block the hepatic ASGP receptor and allow the cells bearing surface asialodeterminants to continue to circulate and migrate to the marrow space or to the organ of interest. The glycoconjugates may be administered to the mammal in any time frame

relative to the cells, but in some embodiments, the glycoconjugates are administered prior to administering the cell. The asialoglycoconjugates and the cell may be administered in any suitable route, but in some embodiments, they are administered intravenously to the mammal, and in other embodiments, they are administered parenterally. In embodiments wherein the cell is targeted to the lungs, the methods feature administering the cell to the mammal in a saline or serum albumin-saline solution. In some embodiments wherein the hematopoietic stem cell is targeted to the heart, the methods feature administering an asialoorosomucoid, and administering the cell to the mammal. In other embodiments wherein the mesenchymal stem cell is targeted to the heart, the methods feature administering an orosomucoid, and administering the cell to the mammal. In embodiments wherein the hematopoietic stem cell is targeted to the liver, the methods feature administering an orosomucoid and administering the cell to the mammal. in other embodiments wherein the mesenchymal stem cell is targeted to the liver, the methods feature administering an asialoorosomucoid and administering the cell to the to the mammal. In some embodiments, the orosomucoid or asialoorosomucoid is administered in at least two infusions, prior to and after administering the cell to the mammal. The methods according to the present invention are also useful for inhibiting sequestration of a cell in the liver of a mammal even in the absence of targeting the cell to a target organ.

The alpha- (1)- acid glycoprotein (orosomucoid or AAG) is a normal constituent of human plasma (650 ± 215 μg ml-1) which increases in concentration as much as fivefold in association with acute inflammation and cancer, and thus is recognized as an acute phase protein. Orosomucoid consists of a single polypeptide chain, has a molecular weight of 44,100, and contains approximately 45% carbohydrate including 12% sialic acid. It is the most negatively charged of the plasma proteins. Certain of the biological properties of orosomucoid are related to its sialic acid content. Thus, clearance and immunogenicity of orosomucoid are markedly increased on desialylation. The biological functions of orosomucoid are largely unknown. Orosomucoid has the ability to inhibit certain lymphocyte reactivities including blastogenesis in response to concanavalin A, phytohaemagglutinin and allogeneic cells, and these inhibitory effects are enhanced in association with desialylation. It has been reported that unphysiologically large (5-15 mg/ml) amounts of orosomucoid inhibit the platelet aggregation induced by ADP and adrenaline, and there is evidence that a sialic acid-deficient species of orosomucoid appears elevated in several chronic disease states.

D. Gene Therapy

The present invention is also directed to using living cells to deliver therapeutic genes into the body. In some embodiments, the therapeutic gene is a transgene. For example, the delivery cells - a type of stem cell, a lymphocyte, or a fibroblast - are removed from the body, and a therapeutic transgene is introduced into them via vehicles well known to those skilled in the art such as those used in direct-gene-transfer methods. While still in the laboratory, the genetically modified cells are tested and then allowed to grow and multiply and, finally, are infused back into the patient. Alternatively, allogeneic cells that bear normal, endogenous genes can reverse a deficiency in a particular target tissue. Use of cells bearing either transgenes or normal, endogenous genes is referred to herein as gene therapy.

Gene therapy using genetically modified cells offers several unique advantages over direct gene transfer into the body. First the addition of the therapeutic transgene to the delivery cells takes place outside the patient, which allows the clinician an important measure of control because they can select and work only with those cells that both contain the transgene and produce the therapeutic agent in sufficient quantity.

Of the stem cell-based gene therapy trials that have had a therapeutic goal, approximately one-third have focused on cancers (*e.g.*, ovarian, brain, breast myeloma, leukemia, and lymphoma), one-third on human immunodeficiency virus disease (HIV-1), and one-third on so-called single-gene diseases *(e.g.,* Gaucher's disease, severe combined immune deficiency (SCID), Fanconi anemia, Fabry disease, and leukocyte adherence deficiency).

In view of the foregoing, the methods according to the present invention are useful for targeting a gene of interest (either a transgene or an endogenous gene) to a tissue in a mammal by introducing a cell comprising the gene of interest and administering a glycoconjugate to the mammal. Such methods are useful for treating a disease characterized by a deficiency in a gene product in a mammal by administering a cell comprising a functional gene encoding the gene product into the mammal and administering a glycoconjugate to the mammal. Stem cells may be used as a vehicle for delivering genes to specific tissues in the body. Stem cell-based therapies are a major area of investigation in cancer research.

The current invention provides localizing of transfused cells such as stem cells to provide a functional gene to a patient suffering from a disease caused by a lack of that gene. In many instances of genetically based diseases, a low level production of that gene product will effectively ameliorate or cure the disease. By providing the gene that is deficient through transfusion of stem cells from a normal donor into the patient, the stem cells may be directed to localize in an organ or tissue of choice, causing a microchimerization of that patient in that organ

or tissue, from which organ or tissue that gene product can be delivered to the patient. Therefore, the present invention provides the ability to direct the localization of the transfused cells such as allogeneic stem cells that have a stable, normal gene. Such transfused cells then create a stable micro-chimera of the recipient.

Those of skill in the art are aware of the genetic deficiencies causative of a large array of genetically based diseases. Exemplary genes and diseases that can be treated include CTFR protein in cystic fibrosis and proteins associated with coagulopathy in the liver. For example, treatment of Hemophilia A can be accomplished using gene therapy. in such embodiment, a transfusion of such cells as umbilical cord blood hematopoietic stem cells may be administered to deliver an intact normal Factor VIII gene. Alternatively, transformed cells can comprise a normal, wild-type Factor VIII gene. Such cells carrying a functional Factor VIII gene may be directed to localize in the liver, preferably by orosomucoid or asialoorosomucoid perfusion prior to the infusion of the stem cells. The cells transform into hepatocytes and begin secreting Factor VIII into the blood.

Other embodiments of gene therapy according to the present invention include treating Hemophilia B (Factor IX deficiency), and antithrombin III, Protein C, and Protein S deficiencies. While these diseases all involve the blood coagulation system, gene therapy may include treating different tissues, such as muscular dystrophy, cystic fibrosis, and the like.

E. Introducing Transgenes Into Stem Cells

Means for introducing transgenes into cells are well known. A variety of methods for delivering and expressing a nucleic acid within a mammalian cell are known to those of ordinary skill in the art. Such methods include, for example viral vectors, liposome-based gene delivery (WO 93/24640; *Mannino Gould-Fogerite, BioTechniques 6(7):682-691 (1988)*; U.S. Pat No. 5,279,833; WO 91/06309; *Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7414 (1987)*; and Budker *et al., Nature Biotechnology,* 14(6):760-764 (1996)). Other methods known to the skilled artisan include electroporation (U.S. Pat. Nos. 5,545,130, 4,970,154, 5,098,843, and *5,128,257),* direct gene transfer, cell fusion, precipitation methods, particle bombardment, and receptor-mediated uptake (U.S. Pat. Nos. 5,547,932, 5,525,503, 5,547,932, and 5,460,831). See also, U.S. Pat. No. 5,399,346.

Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (Sly), human immunodeficiency virus (HIV), and combinations thereof. *See, e.g., Buchscher et al., J. Virol. 66(5):2731-2739 (1992)*; *Johann et al., J. Virol. 66(5):1635-1640 (1992)*; *Sommerfelt et al.,*

Virol. 176:58-59 (1990); *Wilson el al., J. Virol.* 63:2374-2378 (1989); *Miller et al., J. Virol. 65:2220-2224 (1991)*; *PCT/US94/05700*, and *Rosenburg & Fauci*, in *Fundamental Immunology, Third Edition (Paul ed., 1993))*.

AAV-based vectors are also used to transduce cells with target nucleic acids, e.g., in the *in vitro* production of nucleic acids and polypeptides, and in *vivo* and *ex vivo* gene therapy procedures. *See,* West *et al., Virology 160:38-47 (1987)*; U.S. Patent No. 4,797,368; WO 93/24641; *Kotin, Human Gene Therapy 5:793-801 (1994)*; *Muzyczka, J. Clin. Invst. 94:1351 (1994)* and *Samuiski (supra)* for an overview of AAV vectors. Construction of recombinant AAV vectors are described in a number of publications, including *Lebkowski*, U.S. Pat. No. 5,173,414; *Tratschin et al., Mol. Cell. Biol. (1 l):3251-3260 (1985); Tratschin et al., Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, Proc. Nail. Acad. Sci. USA 81:6466-6470 (1984); and Samulski et al., J. Virol. 63:03822-3828 (1989).*

Retroviral vectors are typically used for cells useful in the present invention. Such vectors may comprise, for example, an HIV-2 packageable nucleic acid packaged in an HIV-2 particle, typically using a packaging cell line. Cell transduction vectors have considerable commercial utility as a method of introducing genes into target cells. In particular, gene therapy procedures, in which the cell transduction vectors of the invention are used to transduce target cells with a therapeutic nucleic acid in an in vivo or ex vivo procedure may be used. Gene therapy provides a method for combating chronic diseases caused by a gene deficiency, infectious diseases such as HIV, as well as non-infectious diseases such as cancer.

Stem cells such as CD34+ stem cells may be used in ex vivo procedures for cell transduction and gene therapy. The present invention utilizes the feature that stem cells differentiate into other cell types in vitro, or can be introduced into a mammal (such as the donor of the cells) where they will engraft in the bone marrow unless targeted to another organ for differentiation. Hence, the present invention extends to directing stem cells to particular organs to regenerate tissue such as to the heart to regenerate cardiac muscle cells, to the lung to regenerate alveoli, and to the kidneys to regenerate tissue and to directing cells such as CD34+ stem cells to an organ to ameliorate a genetic abnormality by providing efficacious amounts of a deficient gene product. Methods for differentiating CD34+ cells in vitro into clinically important immune cell types using cytokines such a GM-CSF, IFN-γ and TNF-α are known (See, *Inaba et al., J. Exp. Med. 176, 1693-1702(1992)*, and *Szabolcs et al., 154:5851-5861(1995)). Yu et al., PNAS 92:* 699-703(1995) describe a method of transducing CD34+ cells from human fetal cord blood using retroviral vectors.

F. Pharmaceutical Compositions

In other embodiments, the present invention provides pharmaceutical compositions comprising a cell and a glycoconjugate of the invention. Exemplary glycoproteins include orosomucoids and asialoorosomucoids. In other aspects, the present invention features kits for treating tissue damage or for delivering a functional gene or gene product to a tissue in a mammal comprising a cell and a glycoprotein. Stem cells generally have been presented to the desired organs either by injection into the tissue, by infusion into the local circulation, or by mobilization of autologous stem cells from the marrow accompanied by prior removal of stem cell-entrapping organs before mobilization when feasible, i.e., splenectomy.

Glycoconjugates may be administered prior to, concomitantly with, or after infusing the stem cells. In some embodiments, an intravenous fluid bag may be used to administer the glycoconjugate in a saline or dextrose solution with and without protein, or serum-free media, including, but not restricted to, RPMI 1640 or AIM-V. In such embodiments, the glycoconjugate may be mixed with the cells in the same bag or in a "piggyback". The glycoconjugate may also be continued after administration of the cells to permit longer systemic circulation times or increased specific organ accumulation. This procedure may be repeated as often as needed for delivering a therapeutic dose of the cells to the target organ. The preparation may be used with little concern for toxicity given data from animal studies demonstrating no side effects at doses of 3-7 mg of glycoconjugate per ml of blood volume (up to 12 mg/mouse).

Administration of cells transduced *ex vivo* can be by any of the routes normally used for introducing a cell or molecule into ultimate contact with blood or tissue cells. The transduced cells may be administered in any suitable manner, preferably with pharmaceutically acceptable carriers. Suitable methods of administering such cells in the context of the present invention to a patient are available, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present invention.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the

blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Parenteral administration is one useful method of administration. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and in some embodiments, can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. These formulations may be administered with factors that mobilize the desired class of adult stem cells into the circulation.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by the vector as described above in the context of *ex vivo* therapy can also be administered parenterally as described above, except that lyophilization is not generally appropriate, since cells are destroyed by lyophilization.

The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The dose will be determined by the efficacy of the particular cells employed and the condition of the patient, as well as the body weight of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side effects that accompany the administration of a cell type in a particular patient. In determining the effective amount of cells to be administered in the treatment or prophyLAKis of diseases, the physician should evaluate circulating plasma levels, and, in the case of replacement therapy, the production of the gene product of interest.

Transduced cells are prepared for reinfusion according to established methods. See, *Abrahamsen et al., J. Clin. Apheresis* 6-48-53(1991; *Carter et al., J. Clin. Apheresis 4:*113-117(1988); *Aebersold et al., J. Immunol. Methods 112:1-7(1988)*; *Muul et al., J. Immunol. Methods 101:*171-1 81(1987) and *Carter et al., Transfusion 27:*362-365(1987). After a period of about 2-4 weeks in culture, the cells may number between 1×10^6 and 1×10^{10}. In this regard, the growth characteristics of cells vary from patient to patient and from cell type to cell type. About 72 hours prior to reinfusion of the transduced cells, an aliquot is taken for analysis of phenotype, and percentage of cells expressing the therapeutic agent.

For administration, cells of the present invention can be administered at a rate determined by the LD-50 of the cell type, and the side effects of the cell type at various concentrations, as applied to the mass and overall health of the patient. Administration can be accomplished via single or divided doses. Adult stem cells may also be mobilized using exogenously administered

factors that stimulate their production and egress from tissues or spaces, that may include, but are not restricted to, bone marrow or adipose tissues. The exemplary glycoconjugates may be administered concurrently, prior to and/or following stem cells mobilization, or at a time when the amount of cells in the peripheral circulation is optimal for the desired therapeutic endpoint.

G. Adoptive Immunotherapy

It has already been shown that intravenously administered LAK cells are sequestered predominantly in the lungs and the liver (Lotze, M. T., et al., The *in vivo* distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF) -Implication for the adoptive immunotherapy of tumors. *J. Immunol. 125*: 1487-1493 (1980) (possibly due to interaction of asialodeterminants on the LAK cell surface with ASGP receptors on the surfaces of endothelial cells, Kupffer cells, and hepatocytes (Kolb et al., 1979; *supra*; Kolb-Bachofen, et al., 1984, *supra*) and that metastatic tumors in these organs can be dramatically reduced by LAK therapy (Rosenberg, 1987, *supra*) . Intravenously injected murine bone marrow cells, neuraminidase-treated lymphocytes, natural killer (NK), and LAK cells all share this same trafficking pattern (Samlowski et al., 1984, *supra*; Samlowski et al., 1985, *supra*; Kolb et al., 1979, *supra*; Kolb-Bachofen, et al., 1984, *supra*; Rolstad, B. et al., Natural killer cell activity in the rat V. The circulation patterns and tissue localization of peripheral blood large granular lymphocytes (LOL), *J. Immunol. 136*: 2800-2808 (1986); Rosenberg,1987, *supra*). Moreover, all these cells have asialodeterminants on their surface. Kradin, R. L., et al., Tumor-derived interleukin-2- dependent lymphocytes in adoptive immunotherapy of lung cancer. *Cancer Immunol. Immunother 24*: 76-85.(1987), have gamma-camera imaged patients that have received ^{111}In-labeled tumor-derived interleukin-2-dependent lymphocytes (derived from metastatic adenocarcinoma of the lung). These T "killer" cells derived from human tumors also migrate to the liver and lungs. Based on this preferential localization in the liver of human LAK cells and their ability to kill hepatocellular carcinoma, Hsieh et al., Lysis of primary hepatic tumors by lymphokine activated killer cells. *Gut 28*:117-124 (1987), have conducted Phase I trials for the treatment of this tumor. It has also been suggested that for treatment of liver tumors that selective administration of LAK cells with IL-2 via a catheter inserted into the hepatic artery should be an effective means of administration which may decrease the magnitude and scope of side effects (Fagan, E. A., et al., Immunotherapy for Cancer: the use of lymphokine-activated killer (LAK) cells. *Gut 28*:113-116 (987))..

Human, rat, and mouse liver have been shown to specifically sequester, trap, or "clear" desialylated serum glycoproteins (eg., asialotransferrin) by recognition of galactose residues made terminal by the removal of sialic acid (i.e., asialogycoproteins) and aged desialylated erythrocytes via high affinity hepatic asialoglycoprorein receptors (Ashwell, G. The role of cell-surface carbohydrates in binding phenomena. *In*: *Mammalian Cell Membranes*, Vol. 4, Butterworth, London, OX (1977); Asbwell, G., et al., Carbohydrate-specific receptors of the liver. *Ann. Rev. Riochem.51*: 531-554 (1982); Harford et al., The hepatic receptor for asialoglycoproteins. *In*: The Glycoconjugates; Vol. 4; Part B (ed. M. I. Horowitz) Academic Press, New York,1982). The human, rat and rabbit ASGP receptors display virtually identical characteristics: specificity, cation requirements, pH optimum, affinity, subunit size, and temperature dependent internalization of the receptor and degradation of the asialoligand (Dunn et al., Low temperature selectivity initibits fusion between pinocytotic vesicles and lysosomes during heterophagy of ^{125}I-asialofetuin by the perfused rat liver. J. *Biol. Chem. 225* 5971-5978 (1980); Schwartz et al., Characterization of the ASGP receptor in a continuous hepatoma line. *J. Biol. Chem 256*:88 8-8881 (1981); Ashwell et al., 1982, *supra*; Mueller et al., Receptor-mediated endocytosis of asialoglycoproteins by rat hepatocytes: receptor-positive and receptor negative endosomes. *J. Cell. Biol. 102*:932-947 (1986). At 5-20°C the ligand receptor complex is not internalized; whereas, at 37° C this complex was internalized and degraded and the receptor recycled to the cell surface undamaged Mueller et al., 1986, *supra*). On average a cell containing 225,000 receptors can internalize approximately 30,000 soluble ligand molecules per cell per minute; each functional receptor can bind and internalize one ligand every 8 minutes (Schwartz et al., 1982, *supra*). Hepatocytes share asialo- or GalNAc/Gal-specific receptors with Kupffer cells, and liver endothelial cells (Kolb-Bachhofen et al., 1984, *supra*) and the hepatoma line, HEPG2 has been well characterized with respect to this receptor (Schwartz et al., 1981, *supra*). There are 150,000 high affinity sites per HEPG2 cell and 500,000 per normal hepatocytes; the K_d of about 7 x 10^9 M is the same for both. Thus, using adherence to cell line such as HEPG2 with a well-characterized asiaioglycoprotein receptor, as an *in vitro* correlate of *in vivo* adherence (as in the EXAMPLES, below) is a cost effective and simple system in which to determine parameters and possible problems that will be encountered in the *in vivo* trafficking studies.

Parenteral administration of asialoglycoconjugates (e,g., asialofetuin) to block asialogycoprotein receptors has been shown to increase the efficiency of bone marrow engraftment 5- to 10-fold by blocking hepatic sequestration of these cells by blocking hepatic ASGP receptors (Samlowski et al., 1984, *supra*). Given that LAK cells have asialodeterminants on their surfaces, as shown by the *in vitro* studies herein (*see* Examples 5-16), then they also

most likely are taken up or sequestered in the liver via the ASGP receptors. This would result in a net loss of circulating numbers of LAK effector cells that would be available to participate in the reduction or lysis of tumors. Sequestered LAK cells might not reach the tumor. According to the present invention, by blocking the hepatic ASGP receptors hepatic sequestration can be prevented. Ultimately, the efficacy of LAK therapy would be improved by eliminating hepatic sequestration of these cells by the intravenous administration of asiaioglycoconjugates or by modification of the LAK cell surface with sialidases or sialyltransferases. This would allow fewer LAK cells or fewer cycles of LAK therapy or even less IL-2 to be used during the therapy, thereby reducing the toxicity associated with LAK therapy.

In theory, LAK therapy should be one of the safest and least toxic therapies in the treatment of cancer; however, it has not met expectations (Rosenberg, 1987, *supra*; Durant, Immunotherapy of cancer: The end of the beginning? *N. EngI. J. Med. 316*:939-940 (1987)). LAK cells have also been shown to kill unmodified normal cells, including normal lymphocytes, endothelial cells, and hepatocytes, by some investigators, but not by others. The present invention improves the efficacy of LAK therapy by increasing the number of circulating "killer" cells and thereby improving the probability that these cells will encounter tumor cells located in the periphery, instead of being primarily sequestered in the liver. Eliminating hepatic sequestration should therefore improve the response rate of LAK therapy for tumors located in organs other than liver. Preventing hepatic sequestration of LAK cells should also decrease the severe toxicity and the liver damage associated with this therapy. In addition, this will also reduce the possibility of permanent damage caused by the autoimmune destruction of the liver parenchyma by trapped lymphocytes (Kolb-Bachofen et al., 1979, *supra*; Anderson et al., Toxicity of human recombinant interleukin-2 in the mouse is mediated by interleukin-activated lymphocytes. *Lab. Investigation 59*:598-612 (1988).

EXAMPLES

Procedures

Intravenous cannulas were placed into the external jugular vein of NOD-SCID mice under anesthesia (Institutional Animal Care and Use Committee protocol #AM87046-07) to enable the efficient delivery of ^{111} In-labeled stem cells i.v. Tylenol elixir was administered by mouth after recovery from anesthesia. Briefly radiolabeled CD34+ cells were taken up in 100-250ul of 5 % human plasma albumin in saline and injected into the cannula and then flushed with 50ul of the albumin-saline. The mice were imaged by nuclear medicine.

Mice: NOD-SCID, female mice (Nonobese diabetic/LtSz-scid/scid) were obtained from the Jackson Laboratory, Bar Harbor, ME at 1-2 months of age. These animals were maintained in microisolator cages in a special isolator room. The air was HEPA filtered, and the animals were changed in a laminar flow hood within the facility. All food, bedding, and water was sterilized. NOD-SCID mice were ideally suited for the study of xenotransplanted tumors and hematopoietic cells and lymphocytes because of their immunoincompetence including greatly reduced NK activity. *See, e.g.* Hogan, et al., *Biology of Blood & Marrow Transplantation 3:*236-246 (1997); Noort, et al., *Bone Marrow Transplantation 22 Suppl 1:*S58-60 (1998).

All administrations of agents or cells were done either i.v. or i.p.

Stem Cells: CD34+ stem cells were isolated from apheresis stem cell collection products derived from deceased cancer patients. They were purified to 95-99% purity using antibody conjugated to CD34 conjugated to magnetic beads (MACS separation columns; Miltenyi Biotec, Auburn, CA and cryopreserved.

Human mesenchymal stem cells (hMSCs; PT-2501) obtained through a FDA monitored paid bone marrow donor program were purchased from Poietics Technologies, BioWhittaker (Walkerville, MD). The cells were thawed according to manufacturer recommendations, resuspended, and radiolabeled in Mesenchymal Stem Cell Basal Medium (MSCBM).

Proteins administered: Orosomucoid (alpha-1 acid glycoprotein) and asialoorosomucoid (ASO) were administered in the following buffer containing 0.16 mM Caprylate. 10 mM TRIS, 150 mM NaCl, pH 7.0.

Anesthesia & analgesia: A rodent anesthesia cocktail of 0.04 ml per 20-30g mouse i.p. (Rodent Cocktail recipe: 1.5 ml of 50 mg/ml ketamine, plus 1.5 ml of 20 mg/ml xylazine, plus 0.5 ml of 50 mg/ml acepromazine) was used. The anesthetic agent, Rodent anesthesia cocktail, was administered i.p. as follows:

1) for surgery- 0.04 ml per 20-30g mouse, and
2) for imaging- 0.02 ml per 20-30g mouse.

Post-surgical Analgesia: Tylenol 60 ul/20g mouse (6.10 mg) was administered by mouth after anesthesia had partially worn off. The analgesic agent was Tylenol by mouth at 60 ul (6.10mg) per 20g mouse immediately after surgery or at the first signs of distress. Xylazine contained in an anesthetic formulation may also act as an analgesic.

Surgical procedure (Standard cannula placement): After anesthetizing the animals as previously described, the threads for suturing a cannula filled with citrate saline were soaked in 70% ethanol. The anesthetized animals were secured with paper tape on the operating platform ventral side up. The area from just below the clavicle to the ear was shaved. The shaven area

was cleaned with Betadine and rinsed with 70% ethanol. A vertical incision was made in the skin of the right neck from the top of the rib cage to the jaw bone to expose the sternocleidomastoid muscle with the external jugular vein just beneath. To clearly expose the operating field, the skin was retracted with wire hooks (secured to small weights). Retraction should not distort the underlying tissue but should stabilize the area for visualization and cannula insertion. The vein was cleared of overlying fat and fascia using microscopic forceps. The circulation in the superior vena cava was cut off using a half a knot of 4 O silk surgical sutures. One side of the thread was secured with a clamped hemostat. A second piece of thread was looped around the bottom of the vein to make a half knot without pulling it tight. This loop was used to secure the cannula once it had been inserted into the external jugular vein. The surface of the vein was nicked with the microscissors. The cannula was inserted into the vein with beveled side up. The cannula was slid down diagonally until the anchor was flush with the wall of the vein and the lower knot tightened. The cannula was tested by pushing saline through it. The lower knot was finished after verifying no leakage. A full knot was tied around the cannula using the top thread. Saline flow in the cannula was monitored. The top thread was used to go under, catching tissues, and a knot was tied over the cannula again with this thread. A full knot was made using an end of the top thread and the bottom thread. This secures the superior and inferior threads over the hub of the cannula to prevent accidental dislodgement. The cannula was clamped off and the syringe removed. The cannula was positioned underneath the skin of the neck and exteriorized just below the occiput at the nape of the neck while rotating the animal (dorsal side up). An autoclip was used to staple the heat shrink part of the cannula in place near the exit. The cannula was cut to a reasonable length (1.5-2.0 inches), and a wire plug was placed into it. The animal was turned over to its original position and the neck closed with autoclip being careful not to puncture the cannula.

Surgical procedure (Da Vinci Microport Vascular System cannula placement): The Da Vinci Microport Vascular System (Da Vinci Biomedical, South Lancaster, MA) is a closed injection route permitting its implantation up to 2 weeks prior to trafficking experiments without loss of patency. The essential difference is that the port is not externalized as before. This eliminates additional risk for contamination and damage to the cannula caused by chewing and scratching.

The incision area was cleaned with Betadine prior to initial cuts. The mouse was then taped (back side up) to the surgery board. An incision 3-4 mm was made. Next, the incision was made on the chest 4-5 mm. A tunnel was made from the back incision to the front incision in order to feed the cannula through the back to the chest. Heparin was pushed through the cannula.

The cannula was then pulled through using the hemostats. The skin was pulled loose from the tissue on the back for placement of the port. The port was sutured down to the tissue in the middle upper neck area. It was sutured in two places using a triple knot tie. Next, the mouse was turned on its back with its chest up. The cannula was then cut at an angle, where at least 1 mm and at most 2 mm of cannula was inserted in the jugular vein. The jugular vein was isolated in the chest after some fat and tissue was pulled away. The arms of the mice were taped down on their sides because that pushes the chest forward and further exposes the jugular vein. Once the jugular vein was isolated, two sutures were placed around it. The top of the vein was tied off enough to slow the flow of blood, but not to completely stop the flow. The lower tie was one to 2 mm from the top, and it was not tightened. The lower tie was used later to hold the cannula in place and to stop excessive bleeding from the jugular vein. Next, a small cut was made in the jugular vein between the two ties, so that the cannula could be fed into the vein. Once the cannula was placed in the vein the lower tie was tightened around the cannula within the vein. Next, the cannula was checked for leaks by running heparin through the cannula. After verifying no leaks, both incisions were closed.

^{111}Indium Oxine Labeling Procedure: ^{111}In-oxine labeling of adult human CD34+ or mesenchymal stem cells (hMSCs) was performed using a modification of the Amersham Healthcare Procedure for labeling autologous leukocytes.

Harvesting for tissues for histopathology: Tissues were harvested after euthanasia. After the 1-hour-image, the organs were harvested and half the organ was fixed in 10% neutral buffered formalin and the other half was frozen in OTC for frozen sections. The images presented herein are from fixed tissues.

Necropsy Procedure For Collection of Mouse Tissues: An initial midline skin incision from the anterior cervical region to the brim of the pubis was made followed by an abdominal incision following linea alba from the sternum to the pubis with a lateral reflection of the abdominal wall by incision following the caudal ribs. The sternum was reflected anteriorly by cutting the ribs at approximately the level of the costochrondral junction, incising the diaphragm and pericardium as needed. Anteriorly, reflection of sternum was extended to include the ventral cervical muscles to expose the trachea. The trachea and esophagus were incised at the mid cervical area and reflected caudally, cutting attachments as necessary to remove the thoracic viscera in toto. Following removal of the thoracic viscera, the entire heart was dissected free and immersed in 10% neutral buffered formalin. After immersion, the heart was massaged lightly with serrated tissue forceps to force fixative into the cardiac chambers. The trachea with attached lung was then immersed in fixative without further dissection. The spleen was

visualized, omental attachments incised, removed and immersed whole in formalin fixative. The stomach and intestinal tract were removed by incising the rectum and reflecting the viscera anteriorly while cutting attachments as necessary. The liver was removed in toto and immersed whole in formalin fixative. The kidneys were removed and immersed whole in formalin fixative. The pancreas was incised from the anterior duodenum and immersed in formalin fixative.

Trimming of Tissues for Paraffin Processing and Microtomy: The heart was placed on the trimming board with the right ventricle on the upperside and the left ventricle on the underside next to the trimming surface. A single upper to lower incision was made through the right ventricle and atrium and great vessels at the base of the heart continuing through the interventricular septum and the left cardiac chambers to achieve two approximately equal halves. Each half was placed into separate embedding cassettes containing fixative saturated foam pads and labeled "heart1" and "heart2". The entire left and right lungs were separated from midline tissues and placed flat on fixative-saturated foam pads in cassettes labeled left and right lung. Liver sections were taken from the right lateral and medial liver lobes and placed into an appropriately labeled cassette. The left lateral and medial lobes were sectioned and handled in a similar manner. The entire spleen was placed in an appropriately labeled embedding cassette and oriented with one long margin down, taking advantage of the curvature to increase initial sectional area. For one kidney, a whole coronal section was taken from the midpoint of the kidney. The remaining kidney was sectioned longitudinally. Both sections were placed in a single cassette. The collected pancreas was placed on formalin-saturated foam pad in an appropriately labeled cassette.

Imaging procedures: Nuclear Medicine. NOD-SCID mice were anesthetized using rodent anesthesia cocktail. Once anesthetized, the mice were placed on a foam hemi-cylindrical mouse positioning device (MPD) and covered with a tube sock. The MPD allows better visual separation of the lungs and liver as compared to placing the mouse on a flat surface. The foam on which the mouse was placed, and the tube sock covering maintained a comfortable temperature permitting longer imaging without additional anesthesia. The MPD was placed on a narrow table between the dual heads of a Siemens E.Cam Gamma Camera and imaged statically or dynamically in 2-D or SPECT. ^{57}Co-Spot Marker is used to mark anatomic positions (nose, tail, cannula, etc.). The data was analyzed using a Siemens ICON system for regions of interest or percent of injected dose (e.g. liver, spleen, heart).

CT imaging: A CT scan was performed (G.E. Medical System High Speed Spiral Tunnel) for tumor assessment and to enable the registration/alignment of the nuclear medicine image with that of the CT in order to determine precise location of injected radiolabeled stem cells using the method described by *Arata L., Clinical Uses for Medical Image Registration: Experiences at Three Hospitals. Proceedings of PACMEDTec Symposium in Honolulu, Hawaii, August 17-21, 1998* and *Nelson, et al., Electromedica 68 (2000) 45-48.* CT scans were performed during a nuclear medicine imaging session while the animals were under anesthesia. Anesthetized animals were transported to CT, either just prior to or immediately after, the nuclear medicine scan. Usually only one CT was done per animal. CT was used to precisely localize the radiolabeled materials anatomically, by fusing the CT image with that of the nuclear medicine SPECT images.

Gamma camera imaging using a Siemens E.Cam dual head gamma camera monitored the *in vivo* trafficking patterns of all human stem cells described in the following examples. Mice were placed on a Mouse Positioning Device (MPD) and placed between the detectors on the imaging platform.

EXAMPLE 1

ASO Administered I.V. Directs Human CD34+ To The Heart

Asialoorosomucoid (ASO)/High Dose HSC: When an infusion of 5.75×10^6 HSC was preceded by 3.3 mg ASO, 77±1% of the infused cells were found in the heart immediately after infusion, 75±5% remained in the heart region at 1.5hr, decreasing to 52±1% at 24 hr.

5.75×10^6 ^{111}In-labeled human CD34+ (hCD34+) peripheral blood stem cells were administered intravenously (i.v.) via an external jugular vein cannula to 2 month old, NOD-SCID, female mice (Non-obese diabetic/LtSz-scid/scid) obtained from the Jackson Laboratory, Bar Harbor, ME. The radiolabeled CD34+ stem cells were administered after pretreatment of the mouse with 3.3 mg of asialoorosomucoid (ASO) i.v. The *in vivo* trafficking patterns were followed by gamma camera imaging using a Siemens E.Cam dual head gamma camera from immediately after injection up to 36 hr postinfusion. Human CD34+ were isolated from apheresis stem cell collection products derived from deceased cancer patients. They were purified to 95-99% purity using antibody conjugated to CD34 conjugated to magnetics beads (MACS) separation columns; Miltenyi Biotec, Auburn, CA and cryopreserved.

Radiolabeled CD34+ stem cells administered after ASO migrated immediately to the heart. Anatomic localization was facilitated by the use of a ^{57}Co -point source positioned at the level of the cannula. Up to 79.2% of the injected dose was located in the heart at 1 .5 hours.

These cells did not migrate to the liver and spleen early in the postinfusion follow up images but could be found in the liver later after 24 hours. However, 51.6-53.2% of the originally injected dose remained in the heart at 24 hours. At 36 hours imaging was conducted with the cannula *in vivo* and with the cannula removed and placed next to the sacrificed animal. These images show that the injected cells were not trapped in the cannula but were actually in the heart.

EXAMPLE 2

O Administered I.V. Enables Human CD34+ Cells To Migrate To The Liver And Spleen But Not To The Heart

Orosomucoid/High Dose HSC: When an infusion of $5.75x10^6$ HSC was preceded by 5.5 mg orosomucoid, 74±3% of infused cells were found in the liver and spleen immediately after infusion, 74±4% of the cells remained in the liver region at 1.5 hr, decreasing to 63±1% at 24 hr.

The preparation and procedures set forth in Example 1 were repeated.

5.75×10^6 ^{111}In-labeled human CD34+ (hCD34+) peripheral blood stem cells were administered intravenously (i.v.) via an external jugular vein cannula to 2 month old, NOD-SCID, female mice (Non-obese diabetic/LtSz-scid/scid) obtained from the Jackson Laboratory, Bar Harbor, ME. The radiolabeled CD34+ stem cells were administered after pretreatment of the mouse with 5.5 mg of orosomucoid (O) i.v.

Mice were imaged and the biodistribution of the radiolabeled hCD34+ cells monitored as described in Example I. Radiolabeled hCD34+ administered after O migrated immediately to the liver/spleen area and remained there until 36 hours. Anatomic localization was facilitated by the use of a ^{57}Co-point source positioned at the level of the cannula. The localization to the liver/spleen region ranged from 76.3% immediately postinfusion to 63.6% at 24 hours. No ^{111}In-labeled cells were found in the region of the heart.

At 36 hours imaging was conducted with the cannula *in vivo* and with the cannula removed and placed next to the sacrificed animal. These images show that the injected cells were not trapped in the cannula. Radioactivity was found at or below the cannula placement, i.e., in the region of the liver/spleen.

EXAMPLE 3

O Enables Hcd34+ Cells To Migrate To The Liver/Spleen Without Significant Migration To The Heart

Orosomucoid/Low Dose HSC: When an infusion of $0.5x10^6$ HSC (one-tenth the previous cell dose) was preceded by 11 mg orosomucoid, 43±2% of infused cells were found in the liver and spleen immediately after infusion, and 40±3% of the cells remained in the liver region at lhr.

The preparation and procedures set forth in Example I were repeated. 0.5×10^6 ^{111}In-labeled human CD34+ (hCD34+) peripheral blood stem cells were administered intravenously (i.v.) via an external jugular vein cannula to 2 month old, NOD-SCID, female mice (Non-obese diabetic/LtSz-scid/scid) obtained from the Jackson Laboratory, Bar Harbor, ME. The radiolabeled CD34+ stem cells were administered after pretreatment of the mouse with 11.0 mg of orosomucoid (O) i.v.

Mice were imaged and the biodistribution of the radiolabeled hCD34+ cells monitored as described above. Approximately 1 hour after infusion, the mice were sacrificed and the organs were harvested, and half of the organ was fixed in 10% neutral buffered formalin. Tissue sections were examined microscopically after immunohistochemical staining for human CD34 and *in situ* hybridization for the visualization of human DNA. Nuclear medicine monitoring for the first ten minutes and 1 hour postinfusion showed that the radiolabeled hCD34+ cells localized to the region of the liver/spleen.

Microscopic examination of the heart after immunohistologic staining for CD34 demonstrated hCD34+ cells in the endocardial blood vessel. A few hCD34+ cells could be seen in the lung in the alveolar septum. Clusters of cells with stem cell morphology could be seen in the hepatic sinusoid. *In situ* hybridization for human DNA clearly showed that hCD34+ cells were not found in the heart muscle or interventricular septum but were present in the lung.

EXAMPLE 4

ASO Followed By O Directs hCD34+ Cells To The Heart And Lung But Not The Region Of The Liver/Spleen

Asialcorosornucoid (ASO) + Orosomucoid/Low Dose HSC: When infused ASO caused HSC to localize in the heart, the protocol was changed to have the ASO bolus chased with a bolus of orosomucoid, to test whether the accumulation in the heart would be maintained. HSC were again concentrated in the heart when an infusion of $0.5x\ 10^6$ HSC was preceded by 3.3 mg ASO, then 5.5 mg orosomucoid. This caused 44±5% of the infused cells to accumulate in the heart immediately after infusion. 37±3% of the infused cells remained in the heart region at 1 hr.

The localization in the heart was the major concentrated signal from the cells, although the percent of infused was reduced from the ca. 75% seen in Example 1.

The preparation and procedures set forth in Example I were repeated. 0.5 x 10^6 ^{111}In-labeled human CD34+ (hCD34+) peripheral blood stem cells were administered intravenously (i.v.) via an external jugular vein cannula to 2 month old, NOD-SCID, female mice (Non-obese diabetic/LtSz-scid/scid) obtained from the Jackson Laboratory, Bar Harbor, ME. The radiolabeled CD34+ stem cells were administered after pretreatment of the mouse with 3.3 mg of ASO i.v. followed by 5.5 mg O i.v.

Mice were imaged and the biodistribution of the radiolabeled hCD34+ cells monitored as described in Example 1. Nuclear medicine monitoring for the first ten minutes and 1 h postinfusion showed that the radiolabeled hCD34+ cells localized to the heart.

Approximately 1 hour after infusion, the mouse was sacrificed and the organs were harvested and half the organ was fixed in 10% neutral buffered formalin.

Microscopic examination of the heart after immunohistologic staining for CD34 revealed clusters of hCD34+ cells in the interventricular septum, and cells within those clusters that were morphologically similar to the stained cells but that were CD34 negative. These images reflected the biodistribution depicted by nuclear medicine studies. The presence of hCD34+ cells in the heart was dramatically demonstrated by in situ hybridization. Both immunohistochemical staining for CD34 and *in situ* hybridization for human DNA demonstrated that the infused stem cells localized to the lung and could be readily seen in the alveolar septa, blood vessels, and other structures. Detection of human DNA revealed the presence of many more cells in the lung and heart than would have been predicted by CD34 staining. No hCD34+ cells or cells morphologically resembling hCD34+ cells were found in liver, spleen or kidney.

EXAMPLE 5

HSC Administered In 5% Human Serum Albumin (Without Orosomucoid Or ASO) Migrated Predominantly To The Lungs.

Plasma Albumin/High Dose HSC: When HSC were administered through the catheter without prior protein infusion, 78±13% of infused cells were found in the lungs at 0 hr, 54±10% at 1 hr, and 50±13% at 12 hr. Histological examination of lungs of mice similarly treated, demonstrated infused cells within the alveolar septa and the vasculature.

2.7x 10^6 ^{111}In-labeled HSC were administered intravenously (i.v.) via a cannula implanted in the external jugular vein of a two-month old, female NOD-SCID mouse in 0.1 ml

saline containing 5% human serum albumin. Mice were imaged and the biodistribution of the radiolabeled hCD34$^+$ cells monitored as described in Example 1.

Radiolabeled HSC, administered in saline containing 5% human serum albumin, migrated immediately to the lungs. Anatomic localization of the labeled cells was facilitated by the use of a ^{57}Co-point source positioned at the level of the cannula exit site below the scapulae and nose. Moreover, the position marker at the cannula was verified to be at the diaphragm by CT whole body scans, transverse and coronal sections. The clip at the cannula exit site served as a landmark. The lungs were visualized below the nose marker and above the cannula marker arid the liver and spleen below the cannula marker. Up to 95.4% of the injected dose was located in the lungs at initial imaging (Table 1). In four mice the values for the lungs ranged from 52.6-95.4% of whole body incorporation for the initial imaging time points. At 1h, HSC were located predominantly in the lungs with some counts visible in the blood circulation. In one mouse at lh some localization was seen below the cannula marker, which may have been liver and spleen; however, the outline was indistinct. At 12 hr in that mouse, radiolabeled CD34$^+$ stem cells were found in the liver/spleen region. However, more than 34.7% (range 34.7-68.5%) of the originally injected dose remained in the lungs of other animals imaged at 12h.

While the localization to the lungs immediately after injection (initial or 0h time points) varied from animal to animal, the percent of the original localization to the lungs remaining at subsequent scans was more constant. Using the dorsal images at lh, 72.1-75.5% of the cells initially localized in the lung were retained in the lung region. Using the dorsal images at 12h, 78%, 72.1% and *50.5%* of the initial lung incorporation remained in the lungs of the three mice imaged.

EXAMPLE 6

Orosomucoid Directs MSC To The Heart

Orosomucoid/Low Dose MSC: When a human mesenchymal stem cell infusion (0.56 x 10^6 cells) was preceded by 11 mg orosomucoid, 68±7% of infused cells were found in the heart at 0 hr, and 61±3% at 1 hr.

MSC were obtained from BioWhittaker, (Poietics Division, cryopreserved PT-2501 >750,000 cells per ampoule) and labeled with ^{111}In as in previous examples, except that the MSC were labeled, washed, and injected in Basal Stem Cell Medium (Poietics) containing 5% human serum albumin (HSA). 0.56 x 10^6 ^{111}In labeled, human mesenchymal stem cells (MSC) were administered via an implanted Da Vinci Microport Vascular System cannula in the external jugular vein of a two-month old, female NOD-SCID mouse in 0.21 ml of basal stem cell medium

containing 5% human serum albumin (HSA). Immediately prior to administration of MSC, 11.0 mg of orosomucoid was administered i.v. in 0.2 ml.

Mice were imaged and the biodistribution of the radiolabeled MSCs cells monitored as described in Example 1. Gamma camera monitoring initially (0 hr) and at 1hr post-infusion showed that the radiolabeled MSC localized to the region of the heart. Region of interest analysis of the images revealed that approximately 61.7-75.5% of the injected radioactivity initially localized to the heart and at 1 hr approximately 58-64% of the infused cells remained in this region. The positions of the cannula, diaphragm, heart, lungs, and liver were verified by CT scans (coronal sections). *In situ* hybridization showed human cells predominantly in the heart, but not the liver.

EXAMPLE 7

ASO Followed By Orosomucoid Directs MSC To The Liver/Spleen

MSC were obtained from BioWhittaker, (Poietics Division, cryopreserved PT-2501 >750,000 cells per ampoule) and labeled with ^{111}In. As in Example 6, the MSC were labeled, washed, and injected in Basal Stem Cell Medium (Poietics) containing 5% human serum albumin (HSA).

Asialoorosomucoid (ASO) + Orosomucoid/Low Dose MSC: This example was designed to compare the trafficking of MSC with HSC (Example 4) at the low cell dose, so the sequential infusion of ASO and orosomucoid used in Example 4 was applied. A human mesenchymal stem cell infusion (0.56x 106 cells) was preceded by 4.3 mg ASO followed by 5.5 mg orosomucoid. 63±5% of the infused cells were found in the liver and spleen at 0hr, and 57±7% at lhr.

$0.56x10^6$ "In-labeled, MSC were administered i.v. in 0.21 ml of basal stem cell medium containing 5% human serum albumin (HSA). Prior to administration of MSC, 0.1 ml containing 4.3mg of ASO, followed by 0.1 ml containing *5.5mg* orosomucoid were administered i.v. The ASO, orosomucoid and MSC were administered via an implanted Da Vinci Microport Vascular system cannula in the external jugular vein of a two-month old, female NOD-SCID mouse.

Mice were imaged and the biodistribution of the radiolabeled MSC monitored as in Example 1. Gamma camera monitoring initially and at 1h post-infusion showed that the radiolabeled MSC localized to the region of the liver/spleen. Region of interest analysis of the initial images revealed that approximately 59.2-66.7% of the injected radioactivity localized to the liver/spleen and at 1h approximately 51.9-61.1% of the infused cells remained in this region.

The positions of the cannula, diaphragm, heart, lungs, and liver were verified by CT scans. In situ hybridization confirmed the gamma camera biodistribution data. Cells containing human DNA were found predominantly in the liver.

EXAMPLE 8

MSC Administered In Either Saline Alone, RPMI-1640 Alone, Or Saline Containing 5% Human Serum Albumin (Without Orosomucoid Or ASO) Migrate To The Lungs And Kidneys

MSC were obtained from BioWhittaker, (Poietics Division, cryopreserved PT-2501 >750000 cells per ampoule) and labeled with ^{111}In. As in Example 6, the MSC were labeled, washed, and injected in saline alone, RPMI-1640 medium (GIBCO BRL, Grand Island, NY) and saline containing 5% human serum albumin (HSA).

Saline alone. -1.14 x10^6 ^{111}In-labeled, MSC were administered i.v. in 0.20 ml of saline alone. MSC were administered via an implanted DaVinci Microport Vascular system cannula in the external jugular vein of a two-month old, female NOD-SCID mouse.

Mice were imaged and the biodistribution of the radiolabeled MSC monitored as in Example 1. Gamma camera monitoring initially post-infusion showed that the radiolabeled MSC localized to the region of the lungs. Region of interest analysis of the initial images revealed that 95% of the injected radioactivity localized to the lungs. At 1 hr, 87% and 4% localized to the lungs and kidneys respectively; at 24 hr, 61% and 13% localized to lungs and kidneys respectively; and at 48 hr, 59% and 14% localized to the lungs and kidneys, respectively.

The positions of the cannula, diaphragm, heart, lungs, and liver were verified by CT scans and a ^{57}Co-Spot Marker is used to mark anatomic positions (nose, tail, cannula, etc.)

RPMI-1640 alone. 1.14 x10^6 ^{111}In-labeled, MSC were administered i.v. in 0.20 ml of RPMI-1640 alone. -MSC were administered via an implanted DaVinci Microport Vascular system cannula in the external jugular vein of a two-month old, female NOD-SCID mouse.

Mice were imaged and the biodistribution of the radiolabeled MSC monitored as in Example 1. Gamma camera monitoring initially post-infusion showed that the radiolabeled MSC localized to the region of the lungs. Region of interest analysis of the initial images revealed that 95 % of the injected radioactivity localized to the lungs. At 1hr,-74% and 7% localized to the lungs and kidneys, respectively, and at 24 hr, 69% and 9% localized to lungs and kidneys respectively.

The positions of the cannula, diaphragm, heart, lungs, and liver were verified by CT scans and a ^{57}Co-Spot Marker is used to mark anatomic positions (nose, tail, cannula, etc.)

Saline containing 5% human serum albumin (HSA). 1.14 x10^6 ^{111}In-labeled, MSC were administered i.v. in 0.20 ml of saline containing 5% HSA. MSC were administered via an implanted DaVinci Microport Vascular system cannula in the external jugular vein of a two-month old, female NOD-SCID mouse.

Mice were imaged and the biodistribution of the radiolabeled MSC monitored as in Example 1. Gamma camera monitoring initially post-infusion showed that the radiolabeled MSC localized to the region of the lungs. Region of interest analysis of the initial images revealed that 94% of the injected radioactivity localized to the lungs. At 1h; 87% and 2% localized to the lungs and kidneys respectively; at 24 hr, 59% and 11% localized to lungs and kidneys respectively; and at 48 hr, 57% and 14% localized to the lungs and kidneys, respectively.

RESULTS

The results of the experiments described above are summarized in Table 1, below.

TABLE 1. Summary of Results of Examples 1-8

Stem Cells / Protein Bolus	% Infused Cells in Lungs	% Infused Cells in Liver/Spleen	% Infused Cells in Heart	% Infused Cells in Kidney
HSC / No Protein	78±3 % at 0 hr 54±10% at 12 hr			
HSC / Orosomucoid		74±3 % at 0 hr 74±4% at 1.5 hr 63±1 % at 24 hr		
HSC / ASO			77±1% at 0 hr 75±5 % at 1.5 hr 52±1 % at 24 hr	
MSC / No Protein	95% at 0 hr 87% at 1 hr 61% at 24 hr [majority at 0 hr *Gao et al., Cells, Tissues, Organs 169:12-20 (2001)*]	[considerable at 48 hr *Gao et al., Cells, Tissues, Organs 169:12-20 (2001)*]		4% at 1 hr 13% at 24 hr 14% at 48 hr
MSC / Orosomucoid			68±7 % at 0 hr 61±3 % at 1 hr	
MSC / ASO		63±5 % at 0 hr 57±7 % at 1 hr		

EXAMPLE 9

The broad objectives of the following experiments was to determine whether human LAK cell populations bind specifically to human hepatoma cells via the ASGP receptor and, if so, how this cell recognition system could be manipulated for lymphocyte cell targeting. The general experimental approach uses similar sialo-asialo-containing plasma proteins in an *in vitro* system mimicking contact with liver cells bearing ASGP receptors, shown in Figure 5.

Adherence of NK/LAK Activity

To Human Minimal Deviation Hepatoma Monolayers

Control cells (no IL-2 treatment) or LAK cells (IL-2-treated human peripheral blood lymphocytes cultured 10U IL-2/ml for 3 days) were adhered to a monolayer of HEP G2 cells for 2 hours at 4°C. The monolayer was pretreated either with asialofetuin (ASF, 200 μg/ml) in media or with fetuin (F, control, 200 μg/ml)) in media. After the Control or LAK cells had been incubated on the monolayer, these cells were then decanted, washed, and tested for cytotoxic capacity in a ^{51}Cr-release assay against the NK-resistant target, Raji. The E:T ratios were 40:1, 20:1, 10:1, and 5:1; the standard error of the means is displayed; the E:T ratio is plotted as the LOG E:T. The results are shown graphically in Figure 6.

CONCLUSION: LAK activity was reduced approximately 50% by incubating these cells on HEPG2 monolayers that had been treated with the control (fully sialated) protein, fetuin (which does not block the ASGP receptor). LAK activity was not removed by incubating these cells on HEPG2 monolayer that had been preheated with asialofetuin (to block the ASGP receptors). LAK or Control preparations that had been incubated with either fetuin or asialofetuin (at 200 μg/ml) for 2h at 40C had identical activity to untreated LAK cell populations. These data support the notion that LAK cells bind to the hepatic ASGP receptor and this binding can be inhibited by blocking this receptor with asialofetuin. The extension of this finding is that hepatic sequestration of LAK cells is at least in part due to the ASGP receptor and that the administration of an asialoglycoconjugate, such as asialofetuin could prevent this entrapment and alter LAK cell trafficking.

Adherence to HEPG2 (ASGP Receptor-Positive, "ASGPR+") and CAKI-2 (ASGP Receptor-Negative, "ASGPR-") at 23° C

This experiment is the same as above , except that the adherence to monolayers was performed at 23° C and not 4° C, for 2 hours. Two monolayers were used : HEPG2, an ASGPR+ cell line, and CAKI-2 (human renal cell carcinoma), an ASGPR— cell line. The effector cell populations that were used were: an untreated 3-day old LAK preparation (LAK) and the same population treated with *Vibrio cholera* neuraminidase (LAK/NS) (30 mU/1 x 10^7 cells/200 µl). The neuraminidase-treated population was the asialopositive lymphocyte control. All cell populations regardless of treatment were greater than 90% viable at the time of assay. Each type of effector population was incubated with media alone, 200 µg/ml ASF or F, as controls. All effectors were assayed on RAJI (LAK-sensitive target; NK-insensitive target) or K562 (NK/LAK-sensitive target); the E:T ratios and the graphic presentation are the same above.

Results. The experiments above gave the following results (see Figures 6-8). For the following discussion, activity on RAJI will be referred to "LAK" activity; activity on K562 will be referred to as "IL-2 activated NK" activity. Some investigators support the idea that NK and LAK recognize and kill targets (fresh and cultured tumor cells) using the same target structures.

(1) Preincubation of effectors, either untreated (LAK) or treated (LAK/NS), with ASF or F, does not affect the ability of the effectors to kill either RAJI or K562 cells.

(2) Neuramninidase treatment enhances LAK activity on RAJI, but does not enhance IL-2 activated NK on K562 (see also Figures 11 & 12)

(3) Adherence to HEPG2 of IL-2 activated NK, with or without neuramninidase treatment, can be partially inhibited by ASF, but not by F at 23° C. (Figures 7 & 8)

(4) Adherence to HEPG2 of LAK activity could not be inhibited with either ASF or F at 23° C. (Figures 9 & 10)

(5) Adherence of LAK activity of the neuraminidase-treated population to HEPG2 could only marginally be inhibited by ASF and not F. (Figure 10)

(6) Adherence to CAKI-2 of IL-2 activated NK or LAK activity could not be inhibited by either ASF or F at 23° C. (Figures 7-10)

CONCLUSIONS: LAK activity (as determined on RAJI targets) and IL-2 activated NK activity (as determined on K562 targets) display different adherence characteristics to HEPG2, an ASGPR+ cell line. At 23° C using ASF, LAK activity adherence to HEPG2 cannot be inhibited; whereas, IL-2 activated NK adherence can be partially inhibited. At 4° C virtually all LAK

activity can be inhibited from adhering to the HEPG2 monolayer by ASF. Adherence to the CAKI-2 (ASGPR-) monolayers cannot be blocked by ASF at 23° C.

These data suggest that adherence to the HEPG2 monolayer is in part mediated by the ASGP receptor and adherence to the CAKI-2 monolayer does not involve this receptor. A working hypothesis is that LAK/NK cells bind to HEPG2 via at least two receptors or recognition structures: 1) the ASGPR, which binds an asialodeterminant on the LAK/NK population and 2) the "LAK" or "NK" recognition structure for a target epitope. The first should be inhibitable by ASF; the second should not be. Binding to CAKI-2 (ASGPR-) should not be inhibited by ASF and is due to a LAK or NK recognition structure binding to the target epitope. This can be further supported by data derived from experiments (see below) in which 250 μg/well of ASF or F were added to the ^{51}Cr-release assay of LAK effectors against the labeled target CAKI-2. Even at a concentration of 1 mg/ml, ASF did not inhibit the ability of LAK to kill CAKI-2 target.

At 23° C the ASGPR recycles and at 4° C it does not, according to Schwartz, et al., Characterization of the ASGP receptor in a continuous hepatoma line. *J. Biol. Chem 256*: 88 78-(1981); Schwartz, A. L., et al., Recycling of the ASGP receptor: biochemical and irnmunocytochemical evidence. *Phil. Trans. R. Soc. Lond. 300*:229-235 (1982). The differences seen in the ability to inhibit adherence may be explained by the temperature dependence of ASGPR recycling and possibly of the LAK recognition structure on the target. At 4° C the LAK:target binding, both by the ASGPR and LAK recognition structure, may have the different affinity for ligand than at 23° C, or possibly at the increased temperature other adhesion molecules are capable of increasing the effector:target interaction. That is, at 4° C the only receptor on HEPG2 that binds LAK with any appreciable affinity is the ASGPR, and this static receptor at this temperature can easily be inhibited by its ligand, ASF. At 23° C more than the ASGPR binds the LAK cell to the target; the ASGPR is recycling in the presence of the ligand, ASF, leaving at least the LAK recognition structure for the target and possibly other secondary adhesion molecules to "cement" the interaction.

These data also suggest that the LAK (as assayed on RAJI) and IL-2 induced NK (as assayed on K562) cells have different affinity receptors or different on/off rates for adherence to HEPG2.

Neuraminidase treatment, in theory, should have increased binding of the LAK cells to the HEPG2 monolayer due to the additional number of asialodeterminants generated by this treatment, but did not. If the number of asialodeterminants was already sufficient to occupy the maximum number of ASGPR on HEPG2, increasing the number of these determinants would not

alter the end-effect. It is also possible there is a specific asialodeterminant that is involved in the binding and that generating more, but irrelevant determinants, will not increase adherence. This suggests the interesting possibility that LAK and the IL-2 activated populations may differ in the ligands that participate in this adherence to HEPG2.

5

LAK Cell Killing of Tumor Targets Is Not Blocked By ASF or F in Pretreatment of Targets or When Added to the ^{51}CR-Release Assay

Because asialodeterminants may play a role in both LAK-target interaction *and* LAK trafficking and liver adherence, it is important to determine whether the use of asialoglycoprotein 10 agents, *in vivo*, to alter trafficking patterns also inhibit cytotoxic activities, rendering such manipulations counterproductive. The preincubation of targets with the addition of asialofetuin or fetuin to the assay, at 250 μg per well, does not block LAK killing of the tumor target, CAKI-2. The LAK preparation was a standard 5-day preparation; however, these data have been replicated with 3-day LAK preparation. (% SPECIFIC RELEASE was determined from 15 quadruplicates whose raw counts per minute differed by less than 10 percent; the assay was a standard 4-hour incubation.)

Table 2. % ^{51}Chromium Release from Caki-2 Targets

AGENT ADDED TO ASSAY	40:1	20:1	10:1	5:1
Media	48	49	23	14
F	58	42	27	16
ASF	52	41	24	13

Spontaneous release (media alone): 2183 cpm.

Spontaneous release (fetuin alone): 2267 cpm.

25 Spontaneous release (asialofetuin alone): 2147 cpm

Total release: 30,600 cpm.

Adherence of NK/LAK Activity After Cell Surface Modification by Neuraminidase or 2,3- and 2,6-Siayltransferases

30 Five-day LAK preparations (20 U/ml; 1 Dupont unit = 44.5 BRMP units) grown in AIM-V (Gibco) were treated (according to the protocols in B. 1.2.3 & 1.2.4) with 3OmU Vibric Cholera neuramindase, 0.48 mU 2,3 -or 10mu 2,6- silalyltransferase per 107 cells. Some of the effectors were incubated in media, 10% PBS in RPMJ 1640 at 23°C for 2 hours. 2 x107 effectors from the untreated LALK, neuramithdase-treated LAK, LAK treated with 2,3 or 2,6 were

suspended in 15 ml media and placed onto either HEPG2 (ASGPR+) or CAKI-2 (ASGPR-) monolayers at 23°C for 2 hours. The flasks were rocked every 15 minutes. The nonadherent cells from these monolayers were decanted and assayed against K562 and RAJI, in addition to the unadhered controls. The results are presented in Figures 11-16. The E:T ratios used were 40, 20,10, and 5 to 1.

Results. See Table 2, above, for Summary. Graphic presentation of this data in Figures 11-16).

(1) IL-2 activated NK (killing K562 targets) is not affected by any cell surface modifications (Figures 11, 12 and 13, top 4 dotted lines); whereas, LAK activity (killing of RAJI) is significantly enhanced by neuraminidase treatment (Figures 12, 15 & 16), but not by 2,3-or 2,6-sialyltransferase treaments Figures 15 & 16) .

(2) No modification of LAK cell surfaces alters adherence to CAKI-2 as compared to untreated LAK (assayed on RAJI) (Figure 15). In contrast, neuraminidase treatment promotes adherence to CAKI-2 of IL-2 activated NK activity (Figure 14, bottom solid line; assayed on K562) as well as 2,3-sialyltransferase treatment (Figure 14, solid line above neuraminidase). Treatment with 2,6-sialyltransferase has no effect on the adherence to CAKI-2 of either LAK or IL-2 activated NK.

(3) No modification of the cell surface dramatically modifies adherence of LAK activity to HEPG2 (Figure 15); however, 2,6-sialyltransferase treatment significantly promotes adherence of IL-2 activated NK (Figure 13, bottom solid); and conversely, 2,3-sialyltransferase treatment significantly prevents adherence of these cells to HEPG2 (Figure 13, top solid line).

CONCLUSIONS: IL-2 activated NK killing and LAK are affected differently by neuraminidase treatment.

IL-2 activated NK adherence to both HEPG2 were altered by cell surface modifications; LAK adherence was not affected by these modifications. This may be due to the amount of sialic acid that can be added to the LAK cell surface which could be determined by dose-response of 2,3- and 2,6-sialyltransferases.

Adherence of IL-2 activated NK to HEPG2 at 23° C could be partially inhibited by ASF (previously reported) and by adding sialic acid with 2,3-sialyltransferase (while 2,6-sialyltransferase treatment promoted adherence).

It is necessary to determine whether adding higher concentrations of ASF (or another asialocompound, e.g., asialoGMl-sugar) as a means of compensating for ASGPR-recycling at 23° C or even at 37° C can prevent adherence of IL-2 activated NK or LAK. Likewise, performing dose-response experiments with 2,3- and 2,6 sialyltransferase to achieve addition of the

maximum amount of sialic acid may allow the dissection of the adherence mechanism because each enzyme adds to different structures: 2,3- to O-linked sugars linked to ser/thr and 2,6-sialyltransferase to N-linked sugars linked to asn. These glycosyltranferases may be equally important in discriminating between the populations responsible for IL-2 activated NK (killing of K562) and those responsible for RAJI killing, LAK.

* * *

All publications, patents, patent applications, and other documents mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications, patents, patent applications, and other documents are herein incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, or other document was specifically and individually indicated to be incorporated herein by reference in its entirety for all purposes. Subheadings are included solely for ease of review of the document and are not intended to be a limitation on the contents of the document in any way.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for delivering a stem cell or lymphoid cell to a target tissue in a mammal comprising the steps of:

(a) administering a glycoconjugate to a mammal;

(b) administering the cell to the mammal.

2. The method of claim 1, wherein the cell is a hematopoietic stem cell.

3. The method of claim 2, wherein the stem cell is obtained from the bone marrow, placenta, muscle, fat or an umbilical cord.

4. The method of claim 1 wherein the lymphoid cell is selected from the group consisting of a natural killer (NK) cell, a lymphokine-activated killer (LAK) cell, a tumor-infiltrating lymphocyte (TIL), a cytotoxic lymphocyte (CTL), and mixtures thereof.

5. The method of claim 1, wherein the glycoconjugate is represented by the general formula P-(S)x-Gal,

wherein P is a peptide residue of a human serum glycoprotein and S is a sugar residue of a human serum glycoprotein; x is an integer from 1 to 100 and Gal is galactose residue.

6. The method of claim 1, wherein the glycoconjugate is selected from the group consisting of an orosomucoid and an asialoorosomucoid.

7. The method of claim 1, wherein the target tissue is a tissue of an organ selected from the group consisting of the heart, the liver, the lungs, and the kidneys.

8. The method of claim 1, wherein the glycoconjugate is administered to the mammal prior to the cell.

9. The method of claim 1, wherein the glycoconjugate and the cell are administered intravenously to the mammal.

10. A method for targeting a hematopoietic stem cell to the heart of a mammal comprising the steps of:

(a) administering an asialo-orosomucoid to the mammal; and

(b) administering the cell to the mammal.

11. The method of claim 10, wherein the cell is administered after the step of administering the asialo-orosomucoid.

12. The method of claim 10, wherein the asialo-orosomucoid is administered via a vessel proximal to the heart.

13. The method of claim 12 wherein the asialo-orosomucoid is administered via a jugular vein.

14. The method of claim 10 wherein the heart of a mammal has suffered ischemic injury prior to administering the asialo-orosomucoid.

15. A method for targeting a mesenchymal stem cell to the heart of a mammal comprising the steps of:

(a) administering an orosomucoid to the mammal; and

(b) administering the cell to the mammal.

16. The method of claim 15, wherein the orosomucoid is administered via a vessel proximal to the heart.

17. The method of claim 16 wherein the orosomucoid is administered via a jugular vein.

18. The method of claim 15 wherein the heart of a mammal has suffered ischemic injury prior to administering the orosomucoid.

19. The method of claim 15, wherein the cell is administered after the step of administering the orosomucoid..

20. A method for targeting a hematopoietic stem cell to the liver of a mammal comprising the steps of:

(a) administering an orosomucoid to the mammal; and

(b) administering the cell to the mammal.

21. The method of claim 20, wherein the cell is administered after the step of administering the orosomucoid.

22. A method for targeting a mesenchymal stem cell to the liver of a mammal comprising the steps of:

(a) administering an asialoorosomucoid to the mammal; arid

(b) administering the cell to the mammal.

23. The method of claim 22, wherein the cell is administered after the step of administering the orosomucoid.

24. A method for targeting a gene of interest to a tissue in a mammal, wherein said gene of interest comprises a transgene, said method comprising the steps of:

(1) introducing a cell comprising the gene of interest to the mammal; and

(2) administering a glycoconjugate.

25. The method of claim 24, wherein the cell is a hematopoietic stem cell.

26. The method of claim 24, wherein the cell is a lymphoid cell.

27. The method of claim 26, wherein the stem cell is obtained from the bone marrow, peripheral circulation or an umbilical cord.

28. The method of claim 24, wherein the glycoconjugate is selected from the group consisting of an orosomucoid and an asialoorosomucoid.

29. A method for treating a disease characterized by tissue damage in a mammal comprising the steps of:

(1) administering a stem cell to the mammal; and

(2) administering a glycoconjugate to the mammal.

30. The method of claim 29, wherein the stem cell is obtained from the hone marrow, peripheral circulation or an umbilical cord.

31. The method of claim 29, wherein the glycoconjugate is selected from the group consisting of an orosomucoid and an asialoorosomucoid.

32. The method of claim 29, wherein the disease is selected from the group consisting of a heart disease, a lung disease, a liver disease a neurological disease and a kidney disease.

33. The method of claim 29, wherein the disease is selected from the group consisting of myocardial infarction, emphysema, cystic fibrosis, hepatitis, stroke, nephritis and microalbuminuria.

34. A pharmaceutical composition comprising a lymphoid cell or a stem cell and a glycoconjugate.

35. The pharmaceutical composition of claim 34, wherein the glycoconjugate is selected from the group consisting of an orosomucoid and an asialoorosomucoid.

36. The pharmaceutical composition of claim 34, wherein the cell is a stem cell.

37. The pharmaceutical composition of claim 34, wherein the cell is a lymphoid cell.

38. An article of manufacture, comprising packaging material and a pharmaceutical composition contained within the packaging material, wherein

the pharmaceutical composition comprises a glycoconjugate that is therapeutically effective for targeting a cell to a desired organ, and wherein

the packaging material comprises a label which indicates that the pharmaceutical composition can be used for targeting a cell to a desired organ.

39. The article of manufacture of claim 38, further comprising additional reagents for making cell suspensions to be administered to a mammal and printed instructions, for use in targeting cells.

40. The article of manufacture of claim 39 further comprising a quantity of stem cells suitable for targeting of such cells in a mammal.

41. The article of manufacture of claim 38, wherein the glycoconjugate is selected from the group consisting of an orosomucoid and an asialoorosomucoid.

42. The article of manufacture of claim 40, wherein the cell is a hematopoietic stem cell.

43. A method to improve the efficiency of an adoptive immunotherapy using a lymphoid cell comprising modification of sialoglycoprotein determinants on the lymphoid cell surface.

44. The method of claim 43 wherein the modification comprises removal of sialic to generate new asialoglycoprotein determininants.

45. The method of claim 44 wherein the modification comprises removal of sialic acid by an enzyme.

46. The method of claim 45 wherein the modification comprises removal of sialic acid by a neuraminidase.

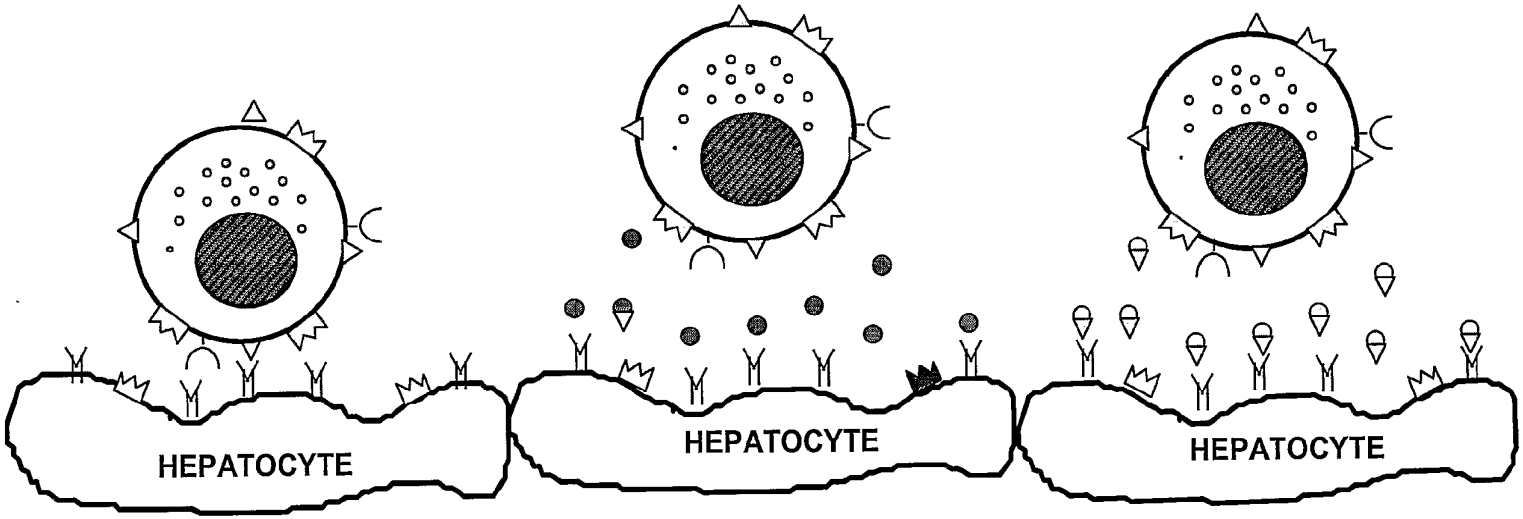
47. The method of claim 43 wherein the modification comprises addition of sialic acid by an enzyme.

48. The method of claim 43 wherein the adoptive immunotherapy is for a liver metastasis or a primary liver tumor. regional administration to the liver of activated lymphocytes.

49. The method of claim 6 wherein the glycoconjugate is administered via a vessel proximal to the organ wherein the target tissue is located.

50. The method of claim 6 wherein the organ is the liver and the glycoconjugate is administered viavia the hepatic artery or portal vein or peripheral vein

MODEL: Liver Entrapment of Bone Marrow Stem Cells & Lymphocytes



KEY TO DIAGRAM

ASIALOGLYCOCONJUGATE
Example:
- asialofetuin (ASF)
- asialoorosomucoid (ASO)

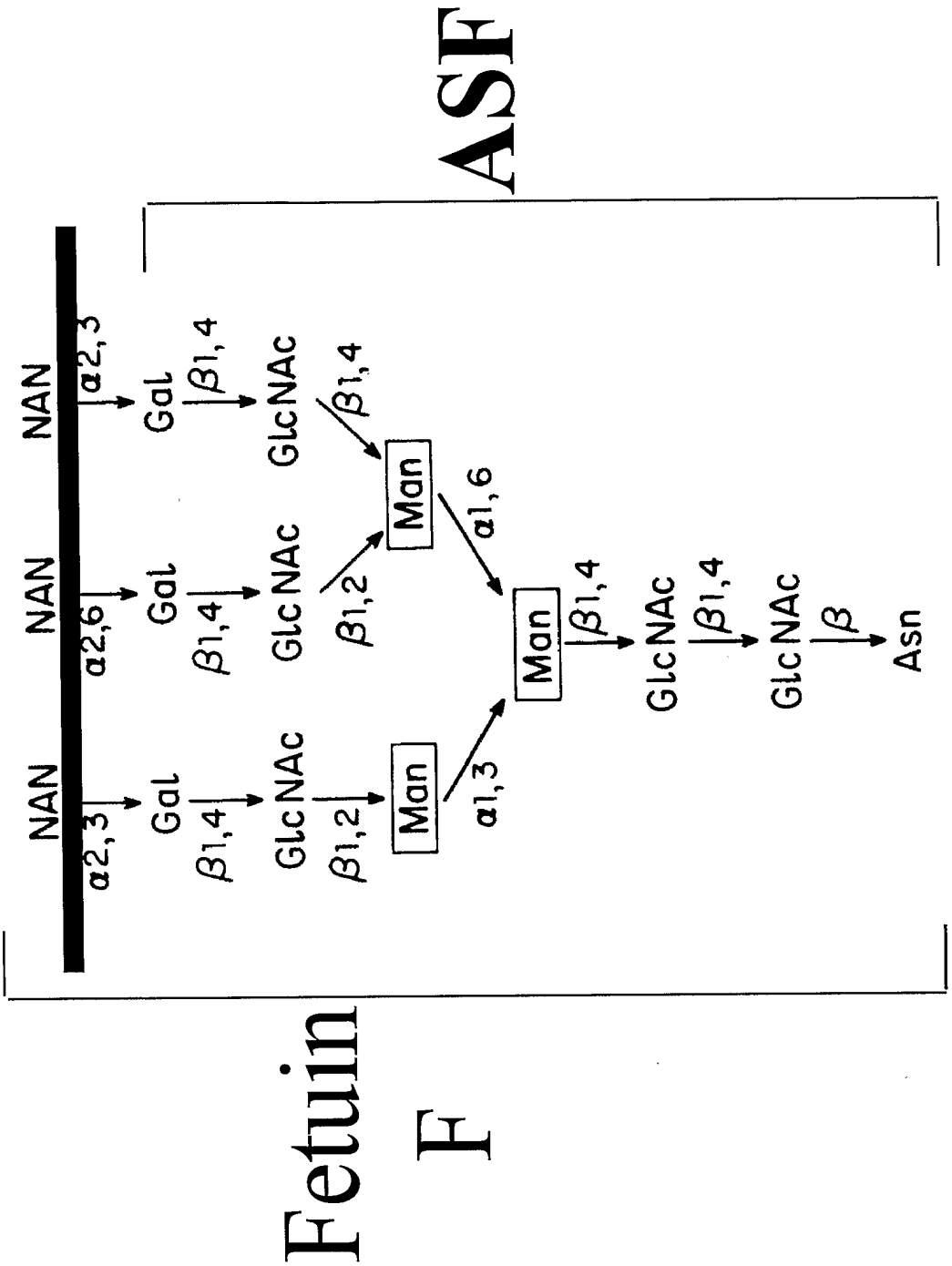
GLYCOCONJUGATE
Example:
- fetuin (F)
- orosomucoid(O)

ASIALODETERMINANT

BLOOD CELL RECEPTOR for tumor epitope or cytokines, etc.

ASIALOGLYCOPROTEIN RECEPTOR (ASGPR)

GENERIC ADHESION MOLECULE


Fetuin
F
ASF
NAN
NAN
NAN
α2,3
α2,6
α2,3
Gal
Gal
Gal
β1,4
β1,4
β1,4
GlcNAc
GlcNAc
GlcNAc
β1,2
β1,2
β1,4
Man
Man
Man
α1,3
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β1,4
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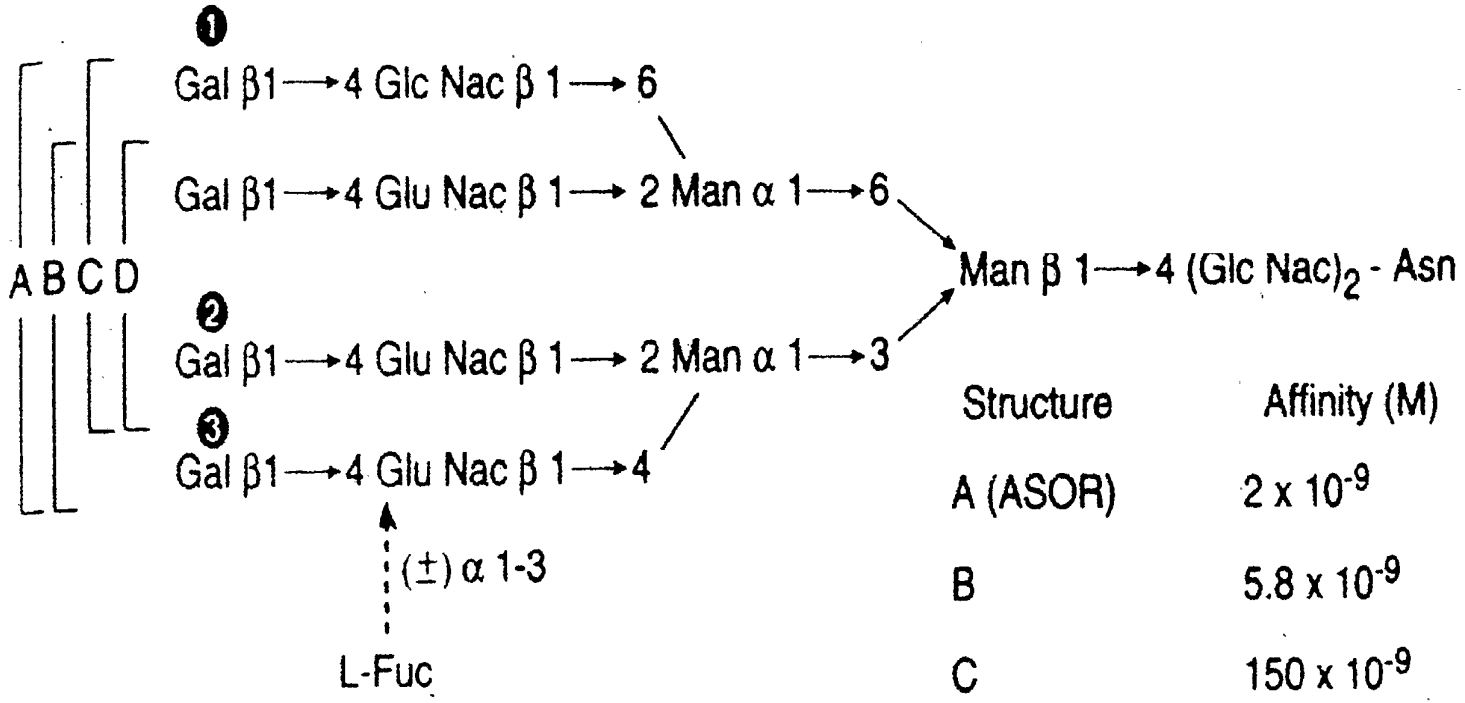
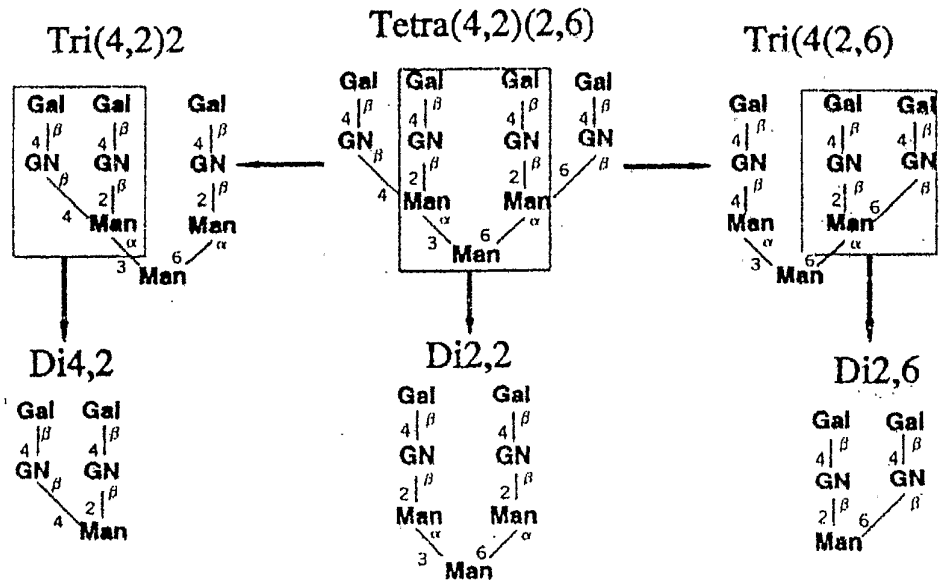
GlcNAc
β
Asn

Comparative Binding Affinities of Asialoglyconjugates for the ASGPR



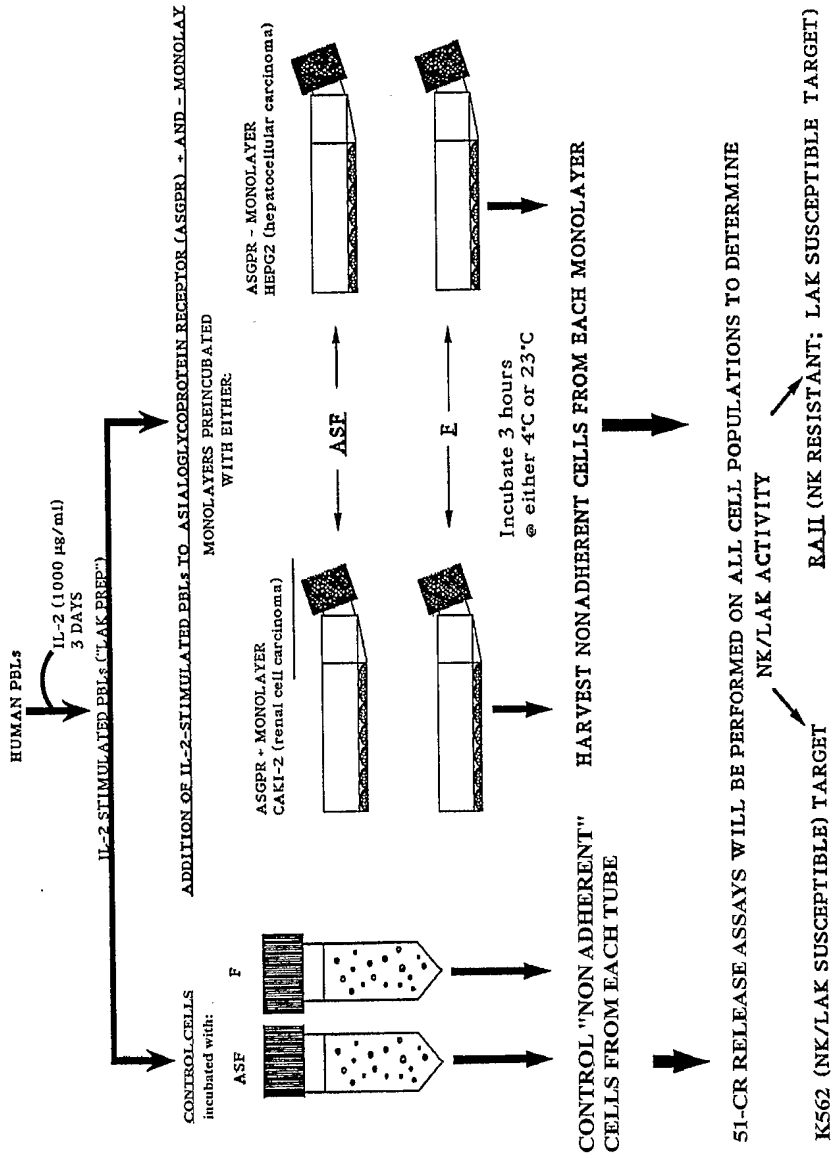
Structure	Affinity (M)
A (ASOR)	2×10^{-9}
B	5.8×10^{-9}
C	150×10^{-9}
D (AsTf)	$49{,}000 \times 10^{-9}$

Saccharide Structure vs. Relative Affinity



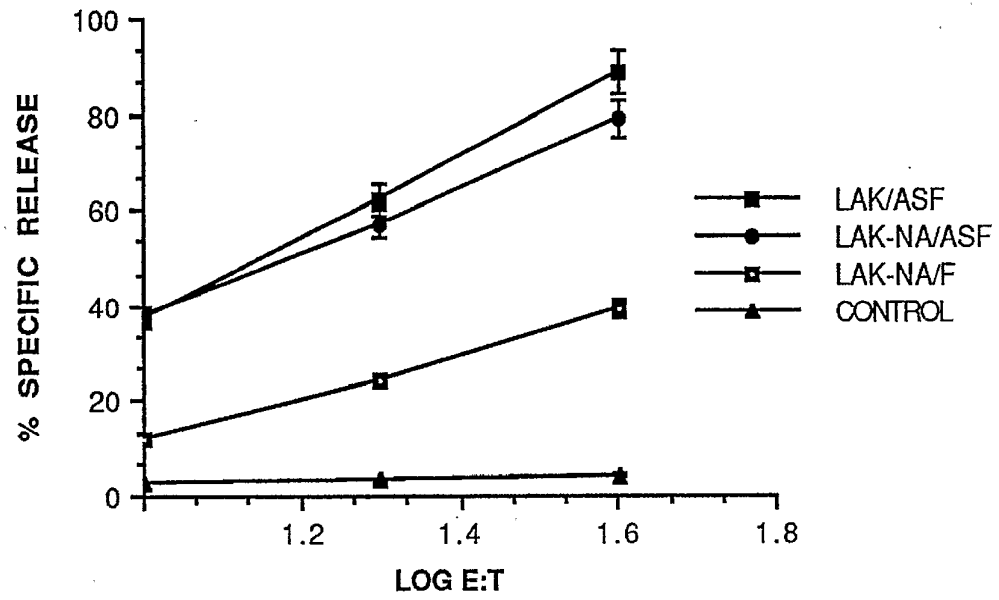
Saccharide Structure	*Relative Affinity*
Galactose	1
Di2,2	20
Di2,6	220
Di4,2	3,700
Tri4(2,6)	6,900
Tri(4,2)2	135,000
Tetra(4,2)(2,6)	295,000

Adherence experiment:



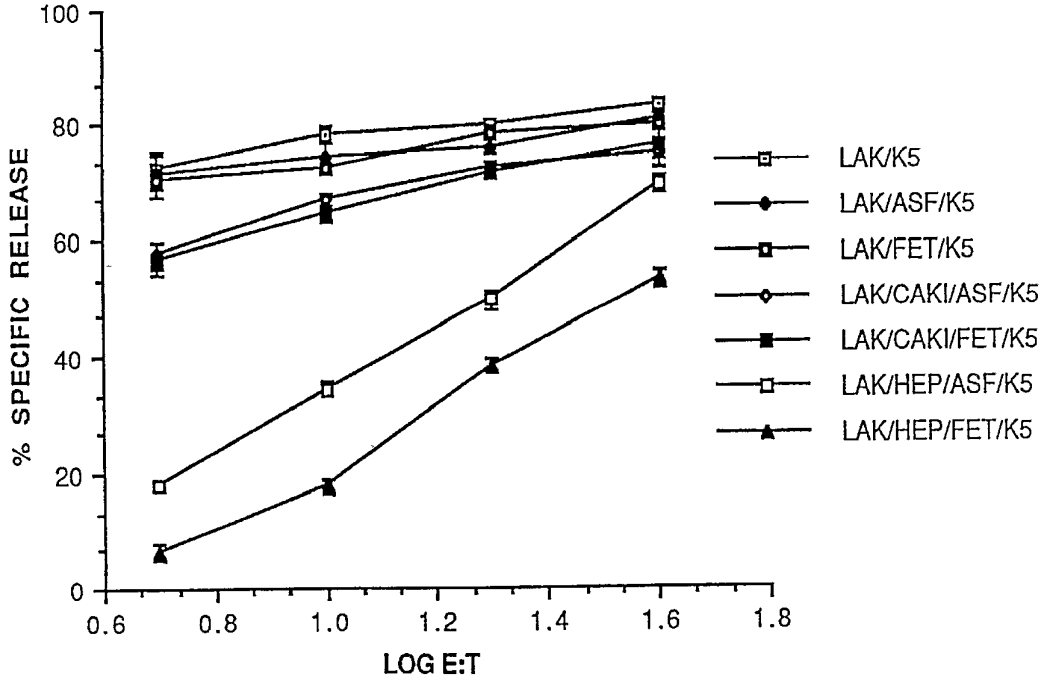
Adherence Experiment: Data

At 4°C, ASF inhibits adherence of LAK activity to HEPG2 monolayer; whereas F does not.



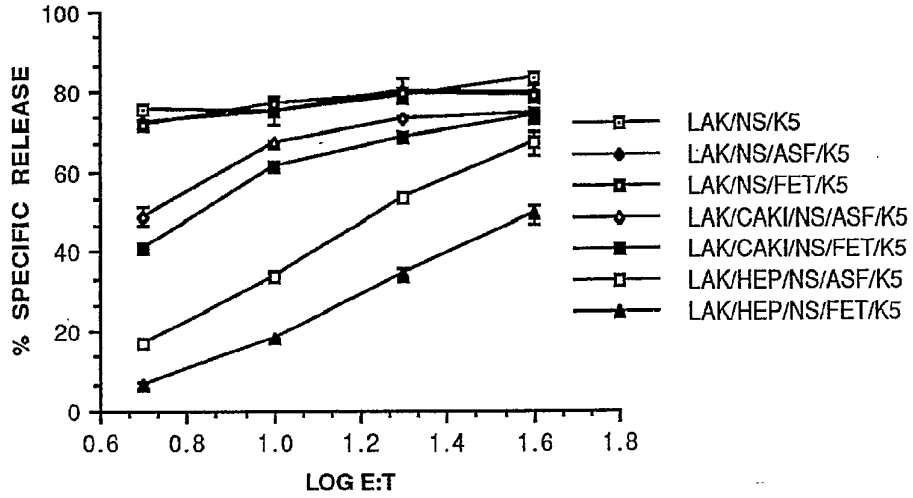
Adherence experiment: Data

At 23°C, ASF partially inhibits adherence of IL-2-activated NK activity to HEPG2 monolayer whereas F does not.

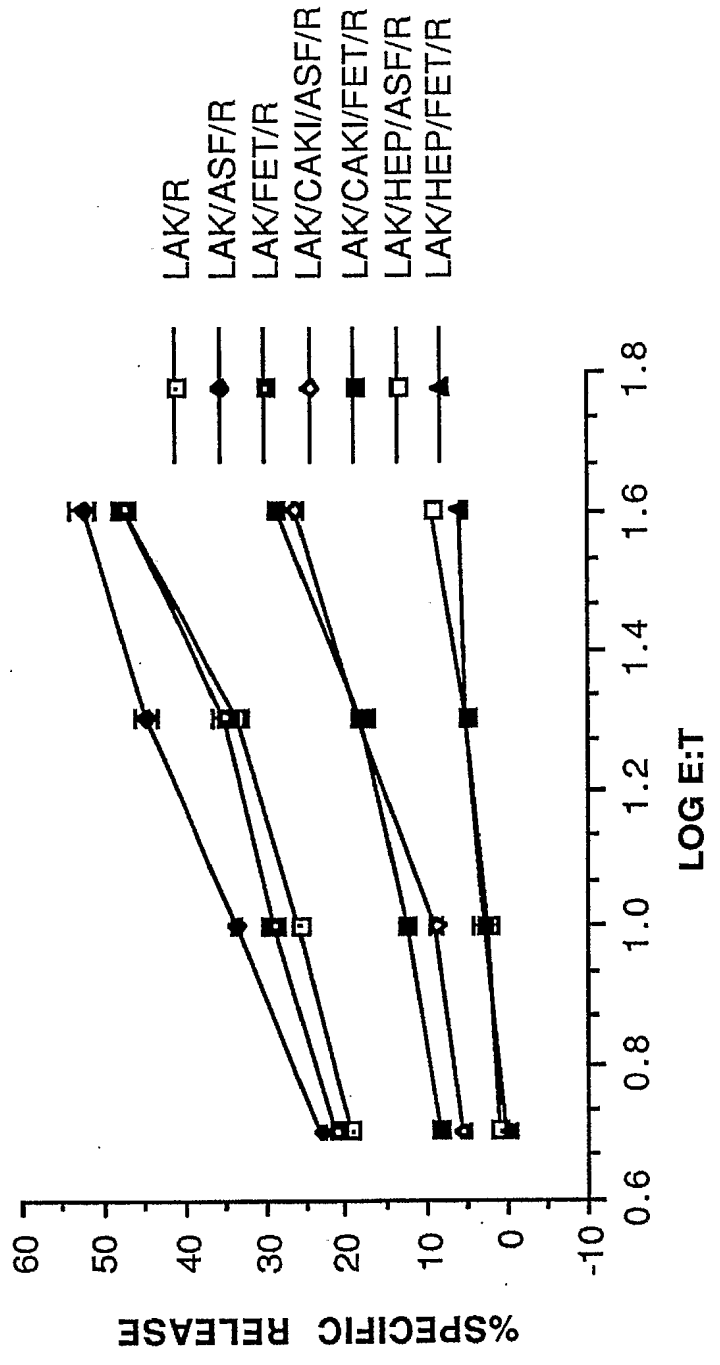


Adherence experiment: Data

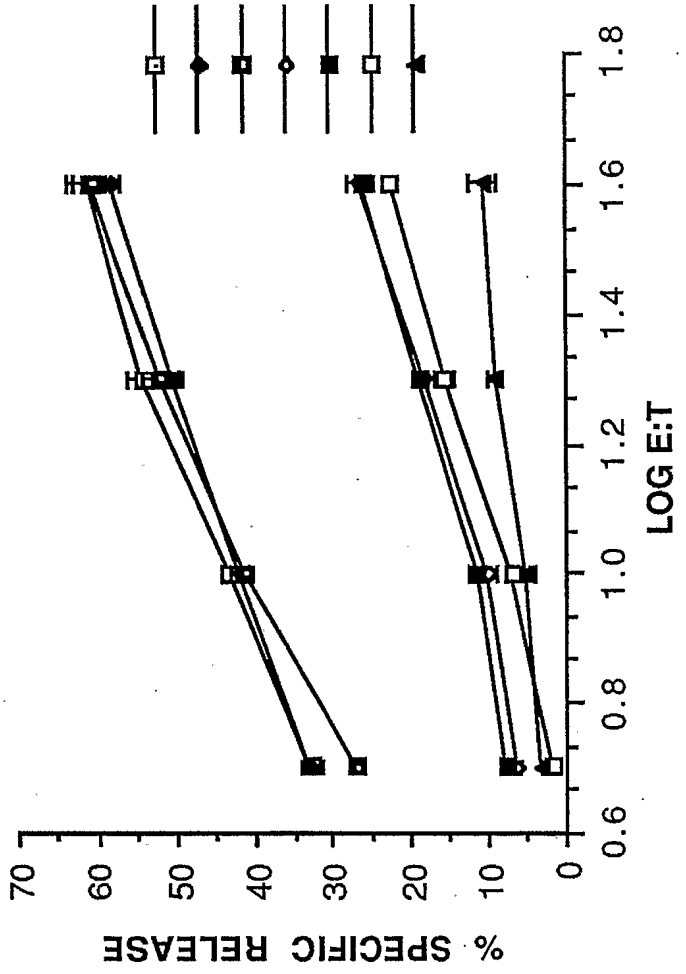
At 23°C, ASF partially inhibits adherence of IL-2-activated NK activity (in the neuraminidase-treated LAK preparation) to HEPG2 monolayer; F does not.



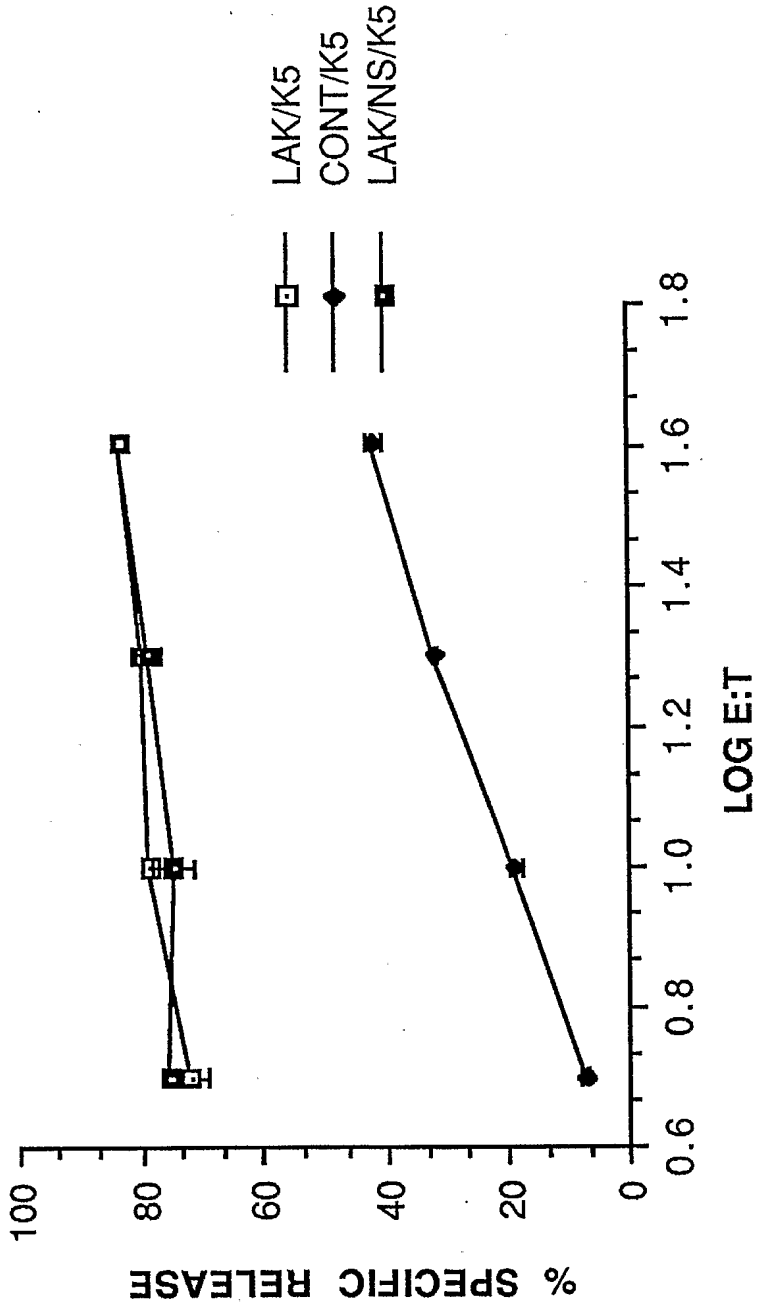
Adherence experiment: Data



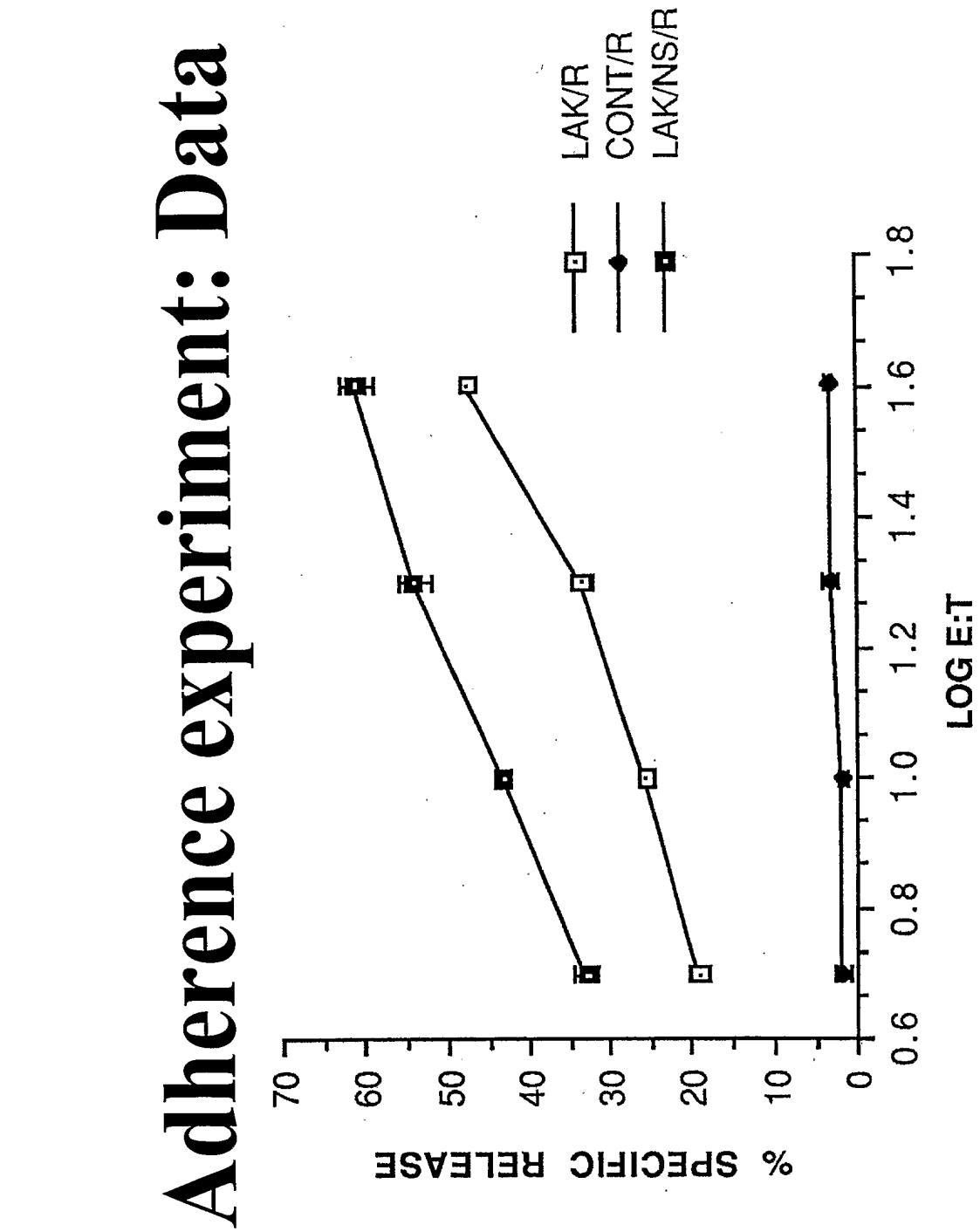
Adherence experiment: Data



Adherence experiment: Data



Adherence experiment: Data



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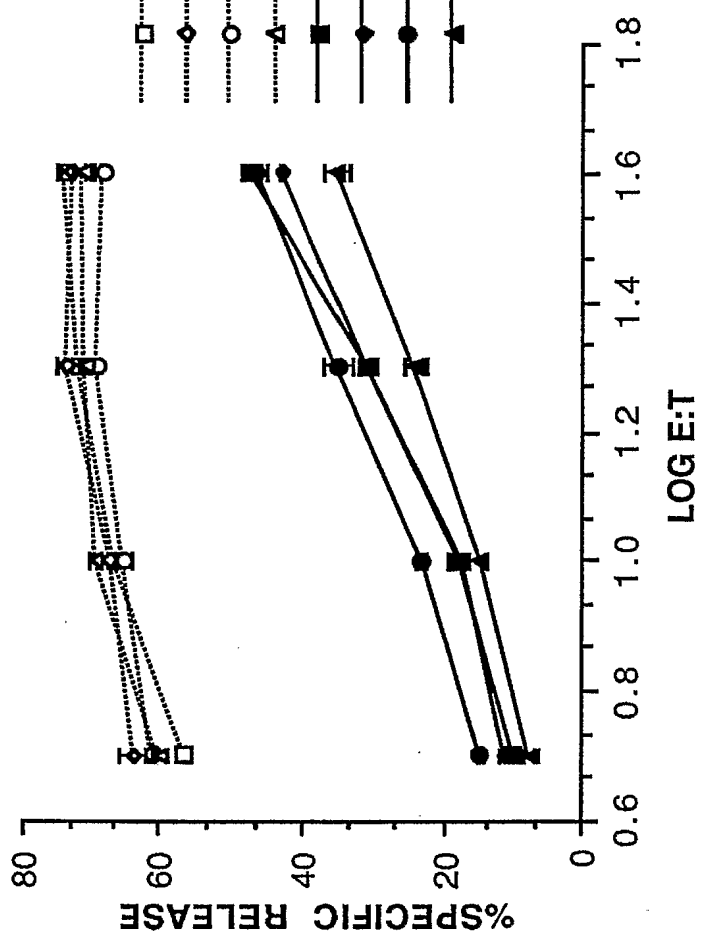
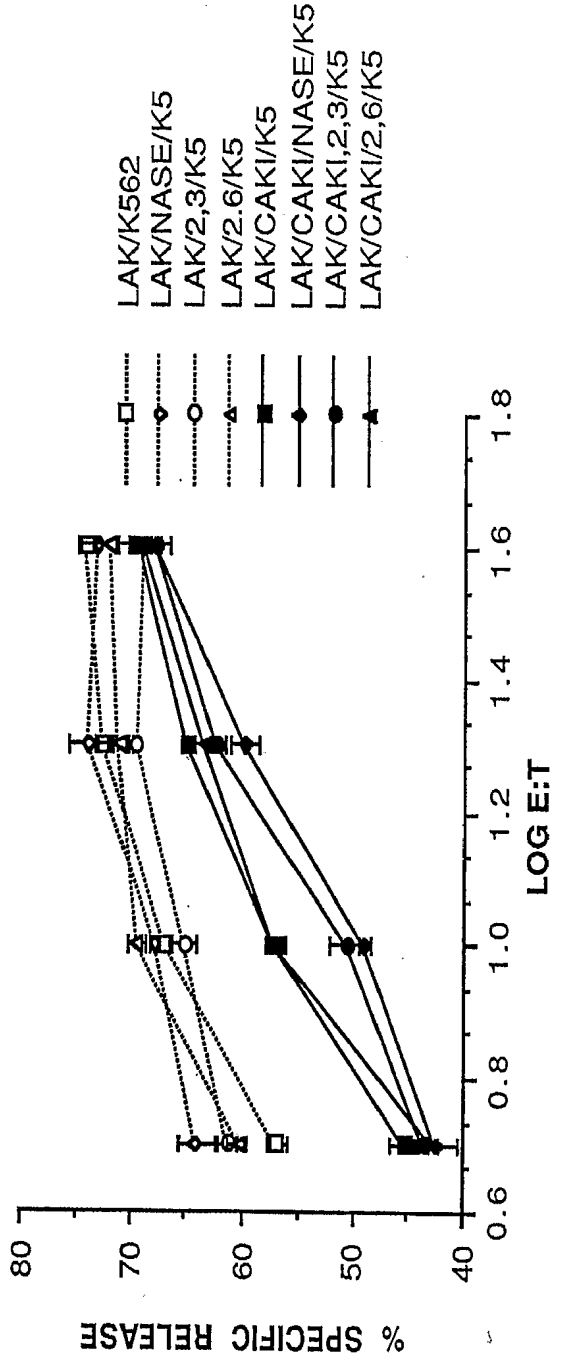
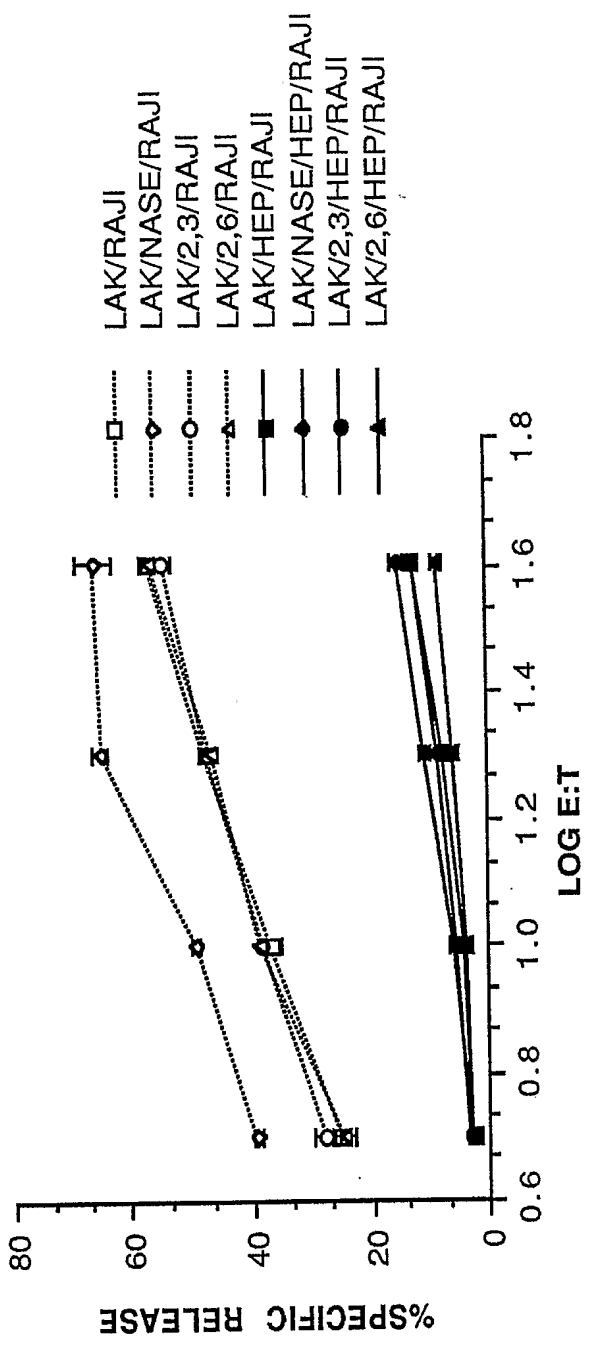


Adherence experiment: Data



Adherence experiment: Data



Adherence experiment: Data